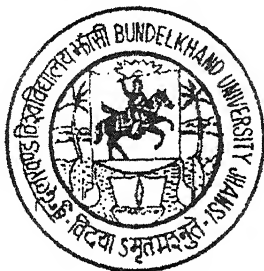


**BIOACCUMULATION AND PATHOLOGICAL STUDIES
RELATED TO HEAVY METAL TOXICITY ON
HETEROPNEUSTES FOSSILIS AND
CYPRINUS CARPIO**



THESIS

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In

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Dedicated To
Late Sri Krishna Kumar
My Father

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*I am pleased to certify that the thesis entitled, "Bioaccumulation And Pathological Studies Related To Heavy Metal Toxicity On *Heteropneustes fossilis* and *Cyprinus carpio*" submitted by Miss Anuja Purwar, M.Sc. embodies the original work carried out by the candidate herself under my supervision and that this work has not been submitted elsewhere for the award of any degree. Certified further that Miss Anuja Purwar has put in over 200 days of attendance in the laboratory to complete the work.*

April 8, 2008


(Dr. Anil Kumar Srivastava)

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SUMMARY

Heavy metal and their salts form an important group of environmental pollutants. Most of the Indian rivers and fresh water streams are seriously polluted by industrial waste. Heavy metals are natural compounds of earth crust. They can not be degraded. Heavy metals enter in nature by erosion and washout processes. By the discharge from industries, from the effluent inflows, amount of heavy metals in water is steadily increased. Heavy metals present in water is dissolved at very low level, since heavy metal compounds have low solubility. They can be bounded easily with water organisms. Once heavy metals are accumulated in aquatic organisms, they can be transfered to upper class of food chain. Heavy metals generally do not degrade and tend to biomagnify in man through food chain. Thus human health eventually is threatened by the consumption of heavy metal contaminated food.

Fish are relatively sensitive to the changes in their surrounding environment. Fish health may thus reflect, and give a good indication of the health status of specific aquatic ecosystem. Heavy metal pollution in Indian aquatic ecosystem, specially river system is a major environment concern.

In this study, the toxic effect of three heavy metals Cadmium, Mercury and Copper have been investigated on the

growth, behavioural abnormalities and histological abnormalities of fresh water fish species *Cyprinus carpio* and *Heteropneustes fossilis*. In this study we have also observed the bioaccumulation of metal, in the selected organs, due to treatment of heavy metals. The total account of the study has been presented in six chapters including conclusion.

Chapter one refers to the introductory part of the research work. In this chapter we have described the possible effect of metals on fish, human and other aquatic life. Metals have been recognized as powerful toxicants. Heavy metals have been observed to cause diseases, behaviour abnormalities, cancer and gene mutation in fish. Physiological and biochemical indices of body have also been found to be effected by heavy metal. Some metals in low amounts are essential for normal body activities but severe ill effects have been observed in their high concentration. Fish form an important source of food and nutrition. Aquatic ecosystem polluted with heavy metal, threaten the aquatic organisms and through the fish as food effect human health directly. Toxicity of heavy metal, contaminate the flesh of fish, decrease their food value and ultimately kill the fish. Hence permissible limits have been prescribed by Indian Toxicological Research Centre for various heavy metal in drinking water.

In chapter two we intended to provide a short overview of heavy metals in aquatic environment and literature review of the effect of metal in fish and human. Heavy metals cause adverse effect on human health. Many diseases have been reported in human beings due to consumption of heavy metal contaminated food and contaminated drinking water. Arsenic causes Black foot disease, which has been endemic in Taiwan. Itai-Itai disease was identified among the populations living in Cadmium polluted area in Japan. Wilson's disease is a great risk for human health that is effected from the over exposure of Copper. Mercury poisoning produced a fatal disease called Minamata disease. Initial symptoms of Minamata disease included numbness of limbs, Lips and tongue, impairment of motor nerve control, deafness, and blurring of vision. This chapter also provides the list of sources and uses of heavy metals separately and effect of heavy metals on fish metabolism such as behaviour abnormality, bioaccumulation, histopathology and growth abnormality. Some metals are naturally found in body and are essential to health, for example, Copper and Zinc. Some metals such as Mercury, Arsenic, Cadmium, Nickel etc. that act as poison, interfere with the enzyme system and metabolism of body. Nutritionally heavy metals are directly antagonistic to the essential trace elements. Heavy metals compete with nutrient elements for binding site of transport and storage.

The chapter three incorporates a detailed description of various material and methods followed during the present investigation. Three metals Cadmium chloride, Mercuric chloride and Copper sulphate have been used for toxicity test. The two fresh water edible fish have been used for metal toxicity tests. In the present experiments *Cyprinus carpio* has been treated with Cadmium chloride and Mercuric chloride and *Heteropneustes fossilis* has been exposed to Copper sulphate solution. Methods for these experiments have followed APHA (1992) and EIFAC (1983). In these studies we have selected five parameters for determining the harmful properties of metals. Acute toxicity test results generally is characterized by median lethal concentration LC50. Lethal concentration LC50 is calculated by parametric procedure, Probit analysis (Finney (1981). Median lethal concentration value for different exposure durations viz. 24h, 48h, 72h, and 96h were ascertained. After determining the LC50 values, the experiment was designed to study the effect of toxic stress caused by chronic heavy metal exposure on selected parameters such as bioaccumulation, Histological abnormalities in selected tissues (Gill, Liver and Kidney), growth and behavioural abnormalities in *C. carpio* and *H. fossilis* due to different concentrations of Cadmium, Mercury and Copper.

Chapter four describes the observations and results of experiments and chapter five deals with the discussion of these observations and analysis of the study. In these chapters we first considered the acute toxicity test that are useful for determining the harmful properties of metals. Acute toxicity test demonstrated the effect in short period of exposure. Median lethal concentration at 24hrs, 48hrs, 72hrs, and 96hrs were estimated by probit analysis (APHA 1992) and Finney (1981). The acute toxicity test are conducted to measure the susceptibility and survival potential of organism to a particular toxic substance. Higher LC50 values of metals expresses less toxicity, because greater concentration of toxic substances is required to produce 50% mortality in organism. LC50 values vary from fish to fish and metal to metal.

The toxicity of Cadmium chloride was studied on *Cyprinus carpio*. The median lethal concentration (LC50) for Cadmium chloride were recorded as 4.5 mg/L, 4.2 mg/L, 3.9mg/L and 3.2 mg/L for 24hrs, 48hrs, 72hrs and 96hrs. 5.0 mg/L concentration was found to be highly toxic which caused 100 % mortality in 120 hrs.

Mercury is comparatively more toxic and median lethal concentration (LC 50) were calculated for different durations to be .46mg/L, .42 mg/L, .40mg/L and .38mg/L for 24hrs, 48hrs, 72hrs, and 96hrs for *Cyprinus carpio* (Linn.). At

.50mg/L all test fish were found dead in 120 hrs. In the present study the 96hrs LC50 for *C. carpio* were 3.2 mg/L and .38 mg/L for Cadmium and Mercury. Mercury and Cadmium both are non essential and poisonous metals. In the present study it has been observed that mercury has low LC 50 value than Cadmium so Mercury is more toxic than Cadmium. In the present investigation the 96hrs LC50 value has been found to be 22.4 mg/L for copper in *Heteropnustus fossilis* and it showed that the *Heteropnustus fossilis* is highly susceptible for Copper.

Behavioural profile showed large differences between *H. fossilis* and *C. carpio* in their swimming movement, feeding and social behaviour. The metals exposed fishes *C. carpio* and *H. fossilis* showed fading in coloration a little and fluctuating responses were reported in their feeding behaviour. Behavioural changes in animal are indicative of internal disturbances of body function and disruption in their metabolic pathway. The present study was conducted to investigate the behavioural abnormalities in fish *Cyprinus carpio* (Linn.) exposed to Cadmium and Mercury treatment and cat fish *H. fossilis* exposed to Copper. It is evident from the observations that behavioural changes in fish *C. carpio* for both heavy metals same have pattern of effect. The lower concentration of both Cadmium and Mercury did not cause any significant changes in behaviour. In the second treatment increase in swimming activity and breathing rate has been

observed and higher dose treatment caused lethargic condition and loss of equilibrium in exposed animal. Behavioural changes in *H.fossilis* have also been investigated. The lower concentration of Copper treatment increases locomotion and opercular movement. The second treatment observed increased locomotion and opercular movement and loss of equilibrium. In higher concentration *H.fossilis* showed lethargy and loss of equilibrium.

All the three metals induced changes in fish growth. Growth fluctuations in animals are due to disturbances of body function and disturbances in metabolic pathway. The present study was conducted to investigate the growth abnormalities in fish *C.carpio* (Linn.) on being exposed to Cadmium and Mercury and cat fish *H.fossilis* (Bloch) on being exposed to Copper treatment. The growth performance in *C.carpio* were noticed after 20 days in different concentrations of Cadmium chloride. In the control group (without cadmium chloride) growth of fish showed highest growth rate (9.27%). In the fish treated with .5 mg/L concentration of Cadmium chloride for 20 days, the fish gained weight by 6.90%. In 2.0 and 2.5 mg/L concentration of Cadmium chloride the loss in weight was recorded to be 1.35% & 1.58% respectively. When *C.carpio* were treated to .10 mg/L concentration of Mercuric chloride, the fish gained weight by 6.48%. In the highest concentration of .25mg/L of Mercury chloride, a loss of 1.17% weight was observed. In control

condition fish gained high percentage of weight (10.48%). When *H.fossilis* (Bloch) was treated with 1.0 mg/L concentration of Copper sulphate for 20 days, the fish gained weight by 5.56%. In higher concentration of 15 and 20 mg/L Copper sulphate the loss in weight was recorded by 1.62% and 2.56%. It was observed that fish growth rate got reduced by increasing the concentration of Copper sulphate. It is evident from the observations, in growth changes in fish *Cyprinus carpio* (Linn.) and *Heteropunestus fossilis* (Bloch) that all heavy metals have same pattern of effects. In comparison to control, metal exposed fishes show slow growth rate. In higher concentration of metal, *Cyprinus carpio* and *H.fossilis* showed negative growth.

An investigation on the effect of heavy metals Cadmium, Mercury and Copper on Gill, Liver and Kidney of *Cyprinus carpio* and *H.fossilis* (Bloch) was carried out in the laboratory. The results showed that degree of distortions of metal in Gill, Liver, and kidney was proportionate to exposure period and concentration of metal. There are several histological alterations noticed in *Cyprinus carpio* exposed to Cadmium and Mercury in the present study, such as hyperplasia, hypertrophy of chloride cells and mucus cells, edema of epithelial cells, clumping of gill filament, aneurism, shortening of secondary lamella and fusion. When *H.fossilis* was exposed to Copper sulphate it showed hyperplasia of primary lamellar epithelium,

hyperplasia of secondary lamellar epithelium, lamellar telangiectasis, hyperplasia of chloride cells and epithelial lifting. In Cadmium and Mercury treated *C. carpio* the liver displayed high prevalence of histological changes, with necrosis, representing the dominant structural alterations. In addition, the hepatocytes of *C. carpio* exposed to heavy metals showed hypertrophy, swelling and nuclear pyknosis. In Copper treated *H. fossilis*, liver showed irregular clumping of cells, and prolonged exposure showed lysis of liver cells.

The fish *C. carpio* has been exposed to Mercuric chloride in different exposure durations for the study of histological alterations in kidney. After 96 hrs, marked abnormal changes in kidney such as glomerular distortions and swelling of epithelial cells have been observed. In prolonged exposure to Mercuric chloride, the swelling of epithelial cells, glomerular distortion, necrosis and degeneration of haemopoietic tissue has been observed. The kidney of fish exposed to Cadmium chloride for 20 days showed glomerular alterations. At 30 days interval with same concentration of metal, kidney showed swelling of epithelial cells and glomerular deterioration and presence of large lipid vacuole. 20 days Copper sulphate exposed *H. fossilis* showed degeneration of glomeruli and proximal tubule. After 30 days kidney showed necrosis in proximal tubules.

The accumulation of metal, Mercury, Cadmium and Copper was also study in gill, liver and kidney. The estimation of Cadmium and Copper has been done by direct air acetylene flame method and Copper estimation has been done by cold atomic absorption method APHA (1992). *Cyprinus carpio* was treated with .32 mg/L Cadmium chloride (10% of 96h LC50) for different durations to investigate the accumulation of metal in gill, liver and kidney the organs were excised from experimental as well as control fish separately and tissues were placed in petry dishes and digested, according to APHA standard methods. Metal concentration in sample were estimated using atomic absorption spectrometer. The mean accumulation of Cadmium was found to be .051 $\mu\text{g/g}$, .038 $\mu\text{g/g}$ and .035 $\mu\text{g/g}$ respectively in gill, liver and kidney after 30 days of exposure. *C. carpio* was treated with Mercury chloride .03mg/L (10% of 96h LC 50) to observe the accumulation of metal in its gill, liver and kidney. The mean accumulation of Mercury was found to be .0047 $\mu\text{g/g}$, .0063 $\mu\text{g/g}$ and .0037 $\mu\text{g/g}$ respectively in gill, liver and kidney 30 days of exposure.

Fish *H. fossilis* (Bloch) were exposed in 2.24 mg/L for different exposure durations to study the accumulation of Copper in the tissues of fish. The mean accumulation of 2.29 $\mu\text{g/g}$, 32.01 $\mu\text{g/g}$, and 2.09 $\mu\text{g/g}$ respectively was observed in gill, liver, and kidney after 30 days of exposure. Present

observation revealed that tissue wise accumulation of each heavy metal varies in *C. carpio* for Cadmium in the order Gill>Liver>kidney, for Mercury in the order Liver>Gill>Kidney and Copper accumulation order in tissue of *H. fossilis* was found as Liver>Gill>Kidney. Mercury is more toxic than Cadmium so Mercury were less accumulated in tissue in comparison to Cadmium. This difference in accumulation may be attributed to the proximity of toxicant medium, the physiological status of tissue and the process of detoxification in the tissue. Gill contained lower level of heavy metal than Liver except that in the case of Cadmium which was lower in Liver then Gill. The concentration of metal was lower in kidney. The different degree of metal accumulation in various tissues depended upon the biochemical characteristic of tissue. Target organ such as liver and gill are metabolically active tissues and accumulate heavy level of metal, as was observed in these experiments. All observations justified the transport of metal in the trace amount of metal from various tissues to kidney.

Chapter, Six includes the final conclusions and future recommendations.

CHAPTER-1
INTRODUCTION

Due to industrialization and population explosion, indiscriminate exploitation of natural resources have increased. Massive amount of domestic waste and effluents are discharged into the water bodies including rivers. Industrial waste containing soup of both inorganic compound (including heavy metal) and organic waste, contaminate the fresh water bodies with a wide range of pollutants. These pollutants not only threatened public health and water supplies, but also damage the aquatic life. Contamination of a river with heavy metals has devastating effect on the ecological balance of the aquatic environment. The diversity of aquatic organisms becomes limited with the extent of contamination (Suziki et. al. 1988). Metals are an integral part of our biosphere, made available naturally, often in minor quantities through weathering of rocks. Anthropogenic activity such as burning of fossil fuels, mining, smelting and discharge of industrial, agricultural and domestic wastes, however have accelerated their accumulation in environment. Studies have shown that fish are able to accumulate and retain heavy metals from their environment and it has been shown that accumulation of metal in tissues of fishes is dependent upon concentration and duration as well as other factors such as salinity, temperature, hardness and metabolism of animals (Pagenkopt 1983; Heath 1987; and Allen 1995).

Once Heavy metals are accumulated by aquatic organism they can be transferred to upper class of food chain. Heavy metals generally do not degrade and tend to biomagnify in man, through food chain. Thus human health eventually is threatened by the consumption of such food. For example Minamata disease, which occurred in Kumamoto, Japan in 1953, was the result of consumption of fish and shrimps, contaminated by methyl mercury from waste water discharge by alkaline factories. According to Tinsli (1982) there are two ways for penetration of heavy metal into the organisms either by direct water absorption or by consuming fish as food. Komarrovskii and Polishtuk (1981) reported larger metal load in the tissue of predatory fish species. The ecological specificity of this pollutant is that there are practically no self-cleaning mechanism known for them when present in water, they pass through the trophic chain of aquatic communities. Heavy metal level in the tissues of aquatic animal is occasionally monitored, because the heavy metal concentration in the tissue reflects, past exposure via environment and food. It can demonstrate the current grave situation of aquatic animals, before the toxicity of metals affect the ecological balance of population in aquatic environment (Canli and Kalay 1998).

Heavy metal can enter the water naturally due to erosion and wash out processes, by discharge of the effluent inflow from industrial waste water, rain water and flow through the urban water way. The amount of heavy metals in water steadily increased. They are present in water in dissolved form only at low levels, since heavy metal compounds have low solubility. Mineral suspension and precipitation substances are able to store heavy metal ions on their outer surface. Heavy metals are neither removed nor detoxified readily by metabolic activity. As a result they accumulate and cause deterioration in quality of environment. Microbial transformation of these metals in sediment may however lead there way in food chain as reported for Mercury, Arsenic, Tin, and Selenium (Khan 2001). Several adverse, reports on metal exposure and toxicity have made human being more conscious all over the world. I.T.R.C. (Industrial Toxicology Research Centre 2000), has prescribed permissible limits of heavy metals for drinking water, are summerised in table -1.

Toxic pollutants such as heavy metals are particularly harmful to animal life. Pollutant cause disease, behaviour abnormalities, cancer, gene mutation, physiological malformation or physical deformation of organism that eventually

ingest to absorb them. Toxicity of metals can contaminate their flesh, decrease their value as food or even kill them. Toxicity of ingested heavy metal has been important to human health issues for decades. Many studies have shown that fish are capable of accumulating high level of metal from contaminated water. This is an important exposure pathway for people, who consume fish grown in contaminated water. The population, most affected by heavy metal toxicity are pregnant woman or very young child (Boon and Soltanpour 1992).

Neurological disorders, central nervous systems and cancer of various body organism in some reports is the effect of heavy metal poisoning (ATSDR 1994; ATSDR 1999a; ATSDR 1999b and ATSDR 2000). Mahaffey et. al. (1981) reported lower birth rate and mental retardation of new born children in some cases where pregnant mother ingested toxic amount of heavy metal. According to Dianne et. al. (1999) metals have been recognized as powerful toxins in many studies. Metal with atomic radii often have important cellular function which depend on the formation of their preferred co-ordination complex with oxygen or nitrogen, the ligands of comparable size for example, to control the quaternary arrangement in structure or catalytic domains of protein (eg. Zinc and Copper in superoxide dismutase)

and in the transport of amino acid with Sodium and electrons by Iron and Copper in cytochrome c oxidase. Metallothionein is low molecular weight protein with about 30% sulphur containing amino acid. Metallothionein is known to be important in the regulation of copper and zinc metabolism and in the detoxification of heavy metals, particularly Calcium (Dianne et. al. 1999).

Some metals are naturally found in the body and are essential to health, Iron for example prevent anemia, Zinc is a cofactor in over 100 enzyme reaction. Some metals such as Mercury, Aluminium, Arsenic, Cadmium, Nickel etc. that act as poison, interfere with the enzyme system and hence effect the metabolic activities of the body. Heavy metal are stable elements (they can not be metabolized by the body), they bioaccumulate and are passed up through food chain to human beings. Heavy metals are taken into body via inhalation, ingestion and skin absorption. Some heavy metals are extremely toxic, even in most minute doses, where as others have low toxicity, even in high doses. Iron can cause several oxidative damage, while Copper may compromise with the liver function. Most metal serve a functional role in the body, for example, selenium is needed in the enzyme activity that restores oxidized glutathione.

Important function of selenium is, its role as a powerful antioxidant in preventing cancer. Some metal have no physiological function, Mercury, Lead, Aluminium are in this group. Even the smallest amount have negative physiological effects. If heavy metal enter and accumulate in body tissue faster than the body detoxification pathway, can deposit in them and gradual built up of these toxins will occur. Heavy metal overload in the adrenal gland, reduce the production of hormones, which cause early aging, process. Heavy metal lead to neurological disease such as depression and loss of thinking power. Human exposure to heavy metal has risen dramatically in last 50 years. This has been due to an exponential increase in the use of heavy metal in industrial processes and product. In modern industrial society there is no escaping exposure to toxic chemical and metals. In general, heavy metal are systemic toxins with specific neurotoxic, nephrotoxic, teratogenic effects. Neurotoxins are substances attracted to nervous system. They are absorbed by nerve endings and travel inside the neuron to the cell body, on their way they disrupt vital functions of cells, such as axonal transport of nutrients, mitochondrial respiration and proper DNA transcription. Heavy metal can directly influence behaviour by impairing mental and neurological function, influencing

neurotransmitter production utilization and altering numerous metabolic body processes.

Heavy metals effect on fish in different ways such as alteration of behaviour. Bioaccumulation of metal in the body of organism effect histological and biochemical alteration in fish. Metal also effect early life stage of fish. Behaviour abnormality in various fish species on the exposure of heavy metal have been reported by several studies. Syed Lal and Shah (2002) reported little behaviour change in low concentration and lethargy and loss of equilibrium in high concentration of Copper exposed fish.

The effect of environmental factors, on accumulation of Copper, has been investigated by Cogun and Kargia (2004) in fish. Geeth et.al. (1996) observed that the exposure concentration and duration effect the accumulation of Copper on *Lepidocephalicathys thermalis*. Heavy metals disturb the homeostasis of the fish due to which metal exposed fish show stress. The stress reaction involve various physiological changes which include alteration in blood composition and immune response. Christensen et. al. (1972) have reported that change in blood of bull head fish occur when exposed to Copper solution.

The 96h LC-50 values for most metals are known, to us. It is very important to determine the concentration of specific metal present in the medium. It may be very small concentration in the aquatic medium. Study has to be made in detail as it causes initial occurrence of cellular damage.

Heavy metals effect on specific vital organs such as liver, gill and kidney. Liver contain highest metal concentration because it is an organ of storage and detoxification of metal (Avenant and Marx 2000). Changes in histological structure of specific vital organs due to exposure of sub lethal concentration of metal in various fishes has been reported by many workers. The body constantly tries to eliminate heavy metals via the available exit routes: the liver, kidney and skin. Detoxification mechanism include acetylation, sulfonation, oxidation etc. The liver is most important in these processes. Here most elimination products are expelled though the bile in to the small intestine and should leave the body via the digestive tract. Alteration of epithelium surface of gill in Copper exposed fish *Catla catla* and *Labeo rohita* has been reported by Ahmad and Munshi (1987). Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular change that may occur in target organs, such as gill and Liver (Dutta 1996). The liver is

detoxification organ and essential for both the metabolism and excretion of toxic substances. Liver has ability to degrade toxic compounds but its regulating mechanism can be overwhelmed by elevated concentration of these compounds and could subsequently result in its structural damage. Heavy metals have the ability to bioaccumulate in the liver and kidney, the target organ of heavy metal pollution, and also the body's detoxification organs.

Fish are an important source of human nutrition. Aquatic ecosystem polluted with heavy metal, may therefore threaten human nutrition and health directly. Toxicity of heavy metals can kill fish, contaminate their flesh and decrease their value as food.

Heavy metals can cause genetic mutation. Heavy metal disrupt metabolic processes. Heavy metal alter prooxidant/antioxidant balance and bind to free sulphydryl groups resulting in inhibition of glutathione metabolism, numerous enzyme and hormone function. According to Rik et. al. (2004) chemically reactive pollutant such as electrophiles, react with different nucleophilic biological molecules. Depending on its electrophilicity, an electrophilic pollutant react with soft

nucleophilies, such as thiol groups in protein and peptides or with harder nucleophiles, such as nucleotides in DNA. They also pointed out that reaction with peptides and proteins interfere with the cellular-reducing capacity through conjugation with glutathione or interfere with enzyme activity, while DNA damage leads to mutation.

Nutritionally, heavy metals action is directly antagonistic to essential trace elements and compete with nutrient elements binding site on transport of strong protein, metalloenzyme, enzyme and receptor which result in disturbance of the metabolism and in balance of nutrient element viz carbohydrate protein / aminoacid, lipids, neurotransmitters and hormone. Ranjanna et.al.(1981) reported enhancement of protein contents due to heavy metal. Cadmium, Lead, Copper, Arsenic, Mercury, & Chromium exposed fish reported decrease in R.N.A. and protein content (Jana and Bandyopadhaya 1987).

Fish are relatively sensitive to changes in their surrounding environment including an increase in pollution. Fish health may thus reflect, and give a good indication of the health status of specific aquatic ecosystem. Early toxic effect of pollution is evident on cellular or tissue level before significant

changes can be identified in fish behaviour or external appearance.

Heavy metal pollution in Indian aquatic ecosystems, especially river system is a major environmental concern. Recent studies on Gandhi sagar reservoir in Chambal river near Nagada and Kota, Khan river near Indore, Kshipra river near Ujjain and lower lake of Bhopal have shown accumulation of metal such as Zinc, Manganese, Copper Nickel, Mercury, and Lead in water. Lead concentration in submerged plant and fish from various sites of river Ganga were observed only in down stream site and in fishes collected at Kanpur (NEERI 1987). Fish population will either be adapted to environmental changes, or may die a slow death. Environmental pollution in the aquatic ecosystem is usually at low level but chronic in nature. Physiological and histological studies with more traditional acute toxicity test, may help one to gain insight into the mode and site of toxic action.

Base-line laboratory studies will provide an essential foundation starting necessary research in this field, where external condition can be controlled and fish can be experimentally exposed to one or more metal known to be present

in natural water. The result of these laboratory studies will be useful in predicting the effects of metal exposure in natural aquatic systems and eventually determine species and metal specific histological lesions and changes. Certain heavy metals are necessary for specific body functions due to their nutritional value. High concentration of these metals can however cause toxic effect within an organism.

1.2 Aim of study :-

The aim of present study has been to evaluate the effect of toxic stress caused by acute and chronic heavy metal exposure on selected parameter such as bioaccumulation and histopathological abnormality in selected tissue (Gill, Liver and Kidney), growth and behavioral changes in *Cyprinus carpio* and *Heteropneustes fossilis* due to different concentration of Cadmium, Mercury and Copper.

Objective I :-

Exposure of fish to different concentration of heavy metals.

Objective II :-

Exposing the fish over various time periods including both short and long term exposure.

1.3 Motivation for work :-

This study can accordingly be summarized in the following statements :-

1. Heavy metal pollution is of great environmental concern and its harmful effect must be investigated.
 2. Controlled laboratory studies are necessary to determine species and metal specific toxicity.
 3. It is necessary to determine the concentration of heavy metal, however minute that concentration may be, that will cause initial structural damage at the histological level .
 4. Short and long term exposure periods may significantly influence the histological damage, bioaccumulation of metal in vital organs, growth and behavioral alteration caused by exposure to heavy metal.
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CHAPTER-2
**LITERATURE
REVIEW**

2.0 General :-

The term heavy metal refers to any metallic element that has relatively high density than that of water. In this chapter we are trying to describe about the work which has been done so far regarding the source, use, and toxicity of heavy metals. Heavy metal are natural component of the earth crust. Anthropogenic source of metal in soil and ground water include the use of commercially available fertilizer and disposal of sewage and waste water. Heavy metals are dangerous because they tend to bioaccumulate in living being . They are taken up and stored faster than they are broken down or degraded, hence accumulation occurs in living beings.

Heavy metal cause adverse effect on human health. Many disease have been reported in human being due to consumption of heavy metal contaminated food and contaminated drinking water. Some heavy metal which are trace metals eg. Copper, Zinc are essential and maintain the metabolism of human body. However their high concentration causes to harm the body of the organism in which they accumulate. Some metal are toxic or poisonous even at low concentration such as Arsenic, Cadmium, Lead and Mercury.

Fish are a major component of most aquatic habitats. Fish is an important potent source of food for human and are key unit in man's natural food web. Fish also serve as environmental indicator. Fish are primary indicator of toxification of Streams and Lakes. Fishes share many physiological properties with mammals and are used in the laboratory and the field by environmental managers and health specialist as an assay tool. Several workers investigated that heavy metals cause diseases, behavioral abnormalities, cancer, gene mutation, physiological malformation or physical deformation of fish that ingest and absorb them. These heavy metals tend to reach the aquatic medium from their sources and take their way to human being through the aquatic life upon which they depend for food. Some of these heavy metal are being described for their sources and their possible role which has been worked out by many scientists world over.

2.1. Arsenic :-

2.1.1. Uses and source :-

The atomic mass of Arsenic is 39. Arsenic has been used from decades as ingredient in pesticides and fungicides. Chromated Copper Arsenate (CCA) is a preservative used to

retard the rotting and deterioration of wood exposed to weathering and insects (ATSDR 2000). Arsenic acid ($\text{As}_2\text{O}_3 + \text{H}_2\text{O}$) is used as weed killer and in leaf desiccation of Cotton plants (Woolson 1973). Arsenic is also used for smelting of ore and in electroplating (Cabb et.al. 2000). Atmospheric fall out from smelting and other manufacturing processes can be significant source of Arsenic in environment. Arsenic is also found in chemical processing plants, drinking water, fungicides, meat and sea food etc. Arsenic occurs naturally in the environment contaminating ground water with inorganic Arsenic. Chatterjee et. al. (1995) reported Arsenic in ground water in six districts of west Bengal. Das et. al. (1995) has found Arsenic concentration in drinking water of West Bengal and also determined the Arsenic concentration in urine, hair, skin-scale and liver tissue (biopsy) of affected people.

2.1.2. Toxicity :-

Arsenic is highly toxic and its acute exposure causes gastrointestinal hemorrhage. Armstrong et.al. (1984) reported that two people in a family of eight, died after consuming drinking water contaminated with Arsenic for one week. After examination it was found that it contained 100 ppm Arsenic(2mg

As/Kg/day, the maximum permiasible limit). Chronic and low exposure of Arsenic causes black-foot disease. Black-foot disease has been found to be endemic in Taiwan. People in area of Taiwan received doses of Arsenic .014-.065 mg/Kg/day in drinking water resulting in black foot disease. Some organizations such as International Agency for Research on Cancer (IARC) Environment Protection Agency (EPA) and National toxicology program (NTP) have classified inorganic Arsenic as human carcinogen.

Arsenic treated fingerling of *Channa punctatus* have shown impaired growth and survivality (Sukla et.al. 1987). They have also reported that RNA content of muscle decreased by 11.5% to 966 ug/g They have also reported that the protein content of muscle decreased by 12.5% to 95.39 mg fresh weight in 7.0 mg/L As⁺³ exposed *Channa punctatus* for 31 days.

2.2. Cadmium :-

2.2.1. Uses and sources :-

Cadmium has an atomic mass 112. It is one of commonest environmental metal poison. Cadmium is rarely found as pure metal in nature. It is generally found associated with Oxygen, or as Chlorides, Sulfates, and Sulphides. Cadmium

is often a by product of extraction of Lead, Zinc and Copper from their respective ores (ATSDR 1999 a), Carbonaceous shale, coal and other fossil fuels are also source of Cadmium. Volcanism is the largest natural source of Cadmium. Anthropogenic source of Cadmium in soil and groundwater include the use of commercially available fertilizers and disposal of sludge as soil amendment (Garcia et. al. 1979; Kosla 1986; Peles et. al. 1998; Barker et. al. 1979). Cadmium is also used in welding and brazing in alloys and in the manufacture of batteries, while its compounds are widely employed as pigments for stabilization in the plastic print industries.

2.2.2. Toxicity :-

Itai-Itai disease were identified between 1940 and 1975 among the populations living in Cadmium polluted area in Japan .In this condition there has been found proximal tubular damage in kidney, mild anemia and a painful fracture of bone. According to Dianne et.al (1999), after absorption, Cadmium is transported in blood and induces synthesis of metalloenzyme, to which it bind subsequently. Metalloenzyme (MT) is released in to circulation, and is filtered by the kidneys and reabsorbed, by endocytosis into the cell of proximal tubules.

Acute exposure to Cadmium generally occur in the workplace, particularly in the manufacturing processes of batteries and color pigment used in paint and plastic, as well as in electroplating processes. Symptoms of acute Cadmium exposure are nausea, vomiting, abdominal pain, and breathing difficulty.

Low level chronic exposure to Cadmium cause adverse health effect including gastrointestinal, hematological, musculoskeletal, neurological and reproductive effect. Main target organ for Cadmium following chronic oral exposure is the kidney (ATSDR 1999a). The united states Department of Health and Human Services (DHHS) has stated that Cadmium compounds may be carcinogenic (ATSDR 1999a).

Cadmium is absorbed by many fish and sea creatures and show toxic effect on them. Some reports show that Cadmium is highly toxic in early life stages of fish (Hwang et. al. 1995). Witeska et. al. (1995) reported significant mortality of Carp eggs (*Cyprinus carpio*) when fish exposed in .01 mg/L Cadmium concentration. Lethal concentration of Cadmium also increased the number of erythrocyte and concentration of hemoglobin in the body of various fish species (Mishra and Srivastava 1980).

Antioxidant deffenss consisting of catalase, superoxide dismutase(SOD), Xanthine oxidase(XOD), Glutathion peroxidase (GPx) and glutathione S-transferase were estimated in liver and kidney of fresh water fish subjected to sub lethal concentration of 5ppm Cadmium chloride.

2.3. Copper :-

2.3.1. Uses and source :-

Copper is an essential substance to human life, but in high doses it can cause anemia, liver, kidney damage, stomach and intestinal irritation. People with Wilson's disease are at great risk for their health that is effected from over exposure to Copper. Copper normally occurs in drinking water from Copper pipes, as well as from algal growth.

Copper is semiprecious metal often used in the electric industries. Toxic effect of Copper compounds are applied as algaecides and fungicides. Copper is toxic even in low amount for all water organisms, such as bacteria, algae, fish prey and fish. Copper can have negative effect on the living beings. Copper is a significant trace element for human metabolism. But high concentration damages health, although usually only temporarily and not chronically. Copper is essential

to humans. The daily requirement of an adult has been estimated at 2.0 mg.

2.3.2. Toxicity :-

Copper is essential for all human being but in high concentration Copper has shown toxic effect on fish as well as on other living beings. Behavioral abnormalities, cancer development, biochemical and hematological changes have been observed on Copper exposed fish. An increase in the levels of haemoglobin and haematocrit concentrations was observed in fish after 96h exposure to 2 and 3 mg/L of Copper (Mishra and Srivastava 1980).

Lower Copper concentration of .49 and .104 mkg/L has been reported to increase the concentration of haemoglobin in blood of brown bullhead (*Ictalurus nebulosus*) after 30 days exposure (Charistensen et.al 1972). The amount of glucose in blood of Indian cat fish (*Hetropneustes fossilis*) increased after 24h exposure to lower concentration of Copper 250µg/L (Singh and Reddy 1990).

Kalele and Dhande (2005) reported ultra structural changes in gill lamellae of *Labeo rohita* exposed for 28 days to

sub lethal concentration of Copper sulphate 1.5 mg/L. Copper ion precipitated on gill secretion, causing death by asphyxiation.

2.4. Lead :-

2.4.1. Uses and source :-

Lead is a naturally occurring heavy metal. It is a part of several ores including its own. Lead is also a product of radioactive decay of Uranium²⁰⁶, Thorium²⁰³ and Actinium²⁰⁷ (Sex and Lewis 1987). It is very much used in the batteries (ATSDR 1999b). Other sources of lead in the environment include automobile exhaust, industrial water, waste water sludge and pesticide.

In India steps have been taken to monitor lead levels in water samples of major national rivers, food items and other possible sources, which may contribute Lead to the ecosystem and human body. Lead level increases in environment due to rapid industrialization and sharp increase in petrol driven vehicular traffic. Sadasivan et.al. (1987), found high lead level in sample water from Kanpur, Bombay and Nagpur . The highest level of Lead was observed 51.02 mg/M³ in the samples collected from Vishakhapatnam urban area. Few samples collected from Uttar Pradesh urban area in the winter season showed Lead

concentration of 26.2 mg/M^3 . Vishakhapatnam's highest concentration of Lead is due to Lead smelting activity. Pandaya et.al. (1983) reported that Lead levels in food sample collected in Ahmedabad city were found to range from .55-1.06, .20-1.05, 1.8-2.6, and .40-1.2 mg/gm in food grains, vegetable, fruits, and Cooked food respectively. Integrated Environmental Programme on Heavy metal (IMPHM) survey in west Bengal, Orissa, and Karnataka, has reported that highest level of Lead was observed in all the food constituents including fish collected from West Bengal.

2.4.2. Toxicity :-

Most of the accumulated Lead is sequestered in bones and the teeth. This causes brittle bone and teeth and weakness in wrists and fingers. High exposure (ie. $>30 \text{ mgPb/Kg/day}$) to Lead causes muscle weakness, cramps and joint pain. Impaired kidney and weakness in immune system can also result from over exposure. Lead exposure is also associated with several neurological effect, such as delayed neurological development and effect general brain function. The united state environmental protection agency classified inorganic Lead as a carcinogen.

Lead is a poisonous metal and it has showed toxic effect in fish. Biochemical, histological, haematological changes have been pointed out by many workers in Lead exposed fishes. Lead exposed fish *Colisa fasciatus* shows reduction in spermatogenic activity and haemorrhage in testes (Srivastava 1987). Gill and Pant (1987) have observed that inorganic Lead disturbs the haematological biochemical profiles of fresh water fish *Barbus conchoni*us. Lead induced biochemical and haematological alteration included a hyperglycemic response at both 30 and 60 days when the respective blood glucose values were 54.4 and 41.8% higher than the control value. The glycogen in fish (*B.conchoni*us) were depleted by 22.5 and 59% in liver after 30 and 60 day respectively . The Lead has been reported to also induce structural impairment. Branchial or renal lesion also result due to lead. In physiological dysfunctions, Lead accumulation varies in different organs of fish. Rogers et.al (2003) found that highest Lead accumulation was observed in Gill followed by kidney, and liver . Branchial Na^+ , K^+ ATPase activity in juvenile trout was inhibited by approximately 40% after 48 h exposure of *Oncorhynchus myddes* to 1 mg/L Lead solution.

2.5. Mercury :-

2.5.1. Uses and source :-

Mercury is most widely found in cinnabar (red sulfide) and other areas containing compounds of Zinc , Tin, and Copper and in rock such as limestone. Mercury is generated by anthropogenic source such as fossil fuel combustion, mining and smelting of ore. Mercury has been used as cathode in electrolysis of sodium chloride solution to produce caustic soda; liquid metallic Mercury is used in extraction of Gold. The agricultural use of Mercury is in the form of insecticide and fungicides. Mercury is also used in electric switches, batteries, thermal sensing instruments, cosmetics, and pharmaceuticals. The ultimate deposition of Mercury takes place in the sediments of Lake, river, and oceans, where inorganic Mercury is readily transformed in to highly toxic organic methyl-mercury through bacterial synthesis and other enzymatic and non enzymatic processes. Organic Mercury rapidly accumulates in the aquatic biota and biomagnifies upward through the aquatic food chain. Mercury has been observed in highest concentration in fish specially the large predatory species.

Natural biological processes can cause methylated form of Mercury to form methylmercury which bioaccumulate over million-fold and concentrate in living organism specially fish. Fish derived from the polluted fresh water is highly contaminated with bioaccumulated Mercury. The major source of methylmercury is contaminated water which comes from industries of manufacture of paper, pulp and plastic product.

Forms of Mercury, Monomethylmercury and Dimethylmercury are highly toxic, and cause neurotoxicological disorders. The main path way for Mercury to human is through the food chain.

2.5.2. Toxicity :-

Mercury is a toxic substance which has no known function in human, biochemistry or physiology and does not occur naturally in living organism. Inorganic Mercury poisoning is associated with tremors, gingivitis and/or minor psychological changes, together with spontaneous abortion and congenital malformation and developmental changes in young children. Primary mechanism for the toxic effect of Mercury is inhibiting the antioxidant enzymes such as super oxide dismutase and

catalase. By inhibition of antioxidative processes Mercury could result to exceed level of free radical, which are particularly responsible to disturb mitochondrial function and nervous system. Mercury inhibits the formation of Thyroid hormone (T_3). Chronic exposure of Mercury has been found to induce peripheral neuropathy, tremor, depression, irritability and sleep disturbances. Mercury can also decrease the production of neurotransmitter. Mercury induced deficiencies has been observed in phenylalanine and tyrosine (precursors to catecholamines and thyroxine) tryptophan (precursor to serotonin) and glutamate (precursor to serotonin) and glutamate (precursor to GABA). Mercury is highly toxic element, the toxic effects on fishes have been observed by several workers. Biochemical, histopathological, hematological abnormalities have been reported in many fishes by several workers. Paulose (2004) pointed out the accumulation of Mercury and methylmercury on *Gambusia affinis* fish when is exposed to sublethal concentration of Mercury. Ramesha et. al. (1997) reported toxicity of Mercury on early life cycle stages of *Cyprinus carpio*. Macleod and Pessah (1973) observed that loss of equilibrium, frequent surfacing and shrinking, burst of erratic swimming and gradual onset of inactivity occurred in rainbow trout *Salmo gairdneri* on

Mercury exposure. Behavioral abnormalities have been attributed to nervous impairment due to blockage of nervous transmission between the nervous system and various effector site (Nilagu 1979. Hilmy et. al.(1987) found that mercury exposed fish shows bioaccumulation and alteration of behaviour of fish *Clarias lazera*.

2.6. Nickel :-

2.6.1. Uses and source :-

Nickel metal is hard magnetic metal with a silver color. It is insoluble in water. It occurs free in meteorite and in ore combined with sulfur, antimony or arsenic. Nickel forms alloys with Copper, Manganese, Zinc, Chromium, Iron, Molybdenum etc. Stainless steel is the widely used Nickel alloy. Element Nickel is used in electroplating, anodizing, Aluminium, casting operation for machine parts and coinage, in the manufacture of acid-resisting Magnetic alloys, magnetic tapes, surgical and dental instrument, Nickel-cadmium batteries, and glass. It is used as a catalyst in the hydrogenation process. Exposure to nickel may occur during mining smelting and refining operations. Nickel may damage the kidney and affect liver function. Nickel is carcinogenic and may damage the developing foetus.

2.6.2. Toxicity :-

The effect of heavy metal Nickel chloride, on biochemical component like glycogen of Gill, foot, mantle, digestive gland and whole body of fresh water bivalve, *Parreysia cylindricol* was studied by Chaudhari et. al. (2002). They reported that significant decrease in total glycogen content of gill, foot mantle, digestive gland and whole body was observed due to pollution stress caused by Nickel chloride. Studies on the histopathological effect of the two different sub-lethal concentrations of Nickel on *Cirrhinus mrigala* fingerlings revealed that these metallic salt is capable of producing severe damage to gill and changes in its cellular levels leading to death of fish (Palaniappan et.al 2003). All these changes finally caused the failure of the respiratory mechanism which resulted the mortality of *Cirrhinus mrigala*.

2.7. Zinc :-

2.7.1. Uses and source :-

Zinc is an essential and beneficial element in human growth. Zinc most commonly enters domestic water supply from deterioration of galvanized iron and dezincification of brass. Zinc is present in most of rocks and weathers out and deposit in soil.

Anthropogenic release is primary source of Zinc in environment. Zinc is released from industrial in waste water effluent. Zinc is used as constituent in several alloys, including brass, bronze and die cast metals. Zinc is used in electroplating, smelting and ore processing (ATSDR 1994).

Zinc is essential element in human diet because it is needed to maintain the proper function of the immune system. It also plays an important role in brain activity of human and help in fundamental growth and development of fetus. The average daily intake of 7-16.3 mg Zn/day is the Recommended Daily Allowance (RDA). For Zinc deficiency some people use Zinc supplements. Zinc is not a human carcinogen.

2.7.2. Toxicity :-

Zinc is essential trace element and in high concentration it causes toxic effect in human being. Long term exposure to high (>85 mg/Kg/day) dose of Zinc causes vomiting, diarrhea, abdominal cramping and in some cases intestinal hemorrhage. High dose of Zinc causes anemia. Zinc displaces iron and Copper in the blood and decreases HDL cholesterol, which can lead to cardiac disease. Zinc showed toxic effect in

human and other living organism such as fishes. Mackay et.al. (1975) and Brooks et. al. (1974) reported the organ specificity of Zinc distribution in fish. High Zinc levels are found in fish kidney and liver (Kroupa and Hartvich 1990). Moor and Ramamurty (1987) reported that Zinc induced negative changes which penetrate fish gill. Gill and skin were main route for metal penetrating from the water.

Zinc effect on early life stage of fish. Zinc can cross the chorion of fish eggs and effect the developing embryo. Somasundaram et.al(1984)found that 2 mg/L Zinc alter egg incubation time and caused eye, jaw and spinal deformity. Williams and Holdway (2000) reported the effect of Zinc on embryo hatchability, larval development, and spinal deformities on Rainbow fish.

Hilmy et. al. (1987) reported that the concentration of haemoglobin and haematocrit increase in *Clarias lazera* and *Tilapia zilli* when these fish were treated with 22mg/L and 32 mg/L Zinc solution for 96 h. Torrер et.al. (1986) reported that Zinc causes an increase in leucocytes count in blood of dog fish (*Scyliorhinus canicula*). Dunier and Siwicki (1992) observed that high concentration of Zinc (up to 1g/l)decreased phagocyte

activity of macrophages in carp (*Cyprinus carpio*) while low concentration on contrary stimulated it. An increase in glucose concentration was observed in rainbow trout after 7 days exposure of this fish to 214 mkg/L of Zinc (Watson and Mc Keown (1976). Syed Lal and Shah (2002) reported that lowest concentration of Zinc (.5 mg/L) caused no visible change in fish behaviour, however with 3.0 mg Zn/L fish tended to swim faster and showed an increased breathing rate. The highest concentration of Zinc 7.0 mg/L resulted in increased lethargy and tendency of loss of equilibrium in fish.

CHAPTER-3
**EXPERIMENTAL
PROGRAMME**

3.1. Material and method :-

3.1.1. Collection of the specimens :-

Healthy specimen of *Cyprinus carpio* and *Heteropneustes fossilis* were collected locally from river Yamuna at Kalpi, Distt. Jalaun, Uttar Pradesh and acclimatized in the laboratory condition for 30 days in large plastic pool containing tap water. During acclimatization they were fed with fish food (tokyu) every day for 3 times a day. Water was renewed after every 240 hours with routine cleaning of aquaria, removing faecal matter, dead fish (if any) or unconsumed food.

3.1.1.1. Morphology of *Cyprinus carpio* (Linn.) :-

The common carp or European carp (*Cyprinus carpio*) is widespread fresh water fish (P-1). Common carp are native to Asia and Europe. It has been introduced into the environments worldwide. It can grow to a maximum length of 5 feet, a maximum weight of over 37.3 Kg. Although they are very tolerant to most of conditions, the common carp prefer large bodies of standing water and soft vegetative sediments. As a schooling fish, they prefer to be in group of 5 or more. They are native in a temperate climate in fresh or brackish water and soft vegetative sediment. The common carp eat a vegetarian diet

of water plants but eventually also take insect, crustaceans or even dead fish.

3.1.1.2 Morphology of *Heteropneustes fossilis* (Bloch):-

Heteropneustes fossilis (Bloch) is commonly called singhi in Hindi, inhabits the fresh water and swampy pools (P-2). Body is elongated and laterally compressed with a great depressed head, measuring about 30 cm in length and its skin without scale. Barbles are long and four pairs. Pectoral fins are strong with poison spine and associated venom gland. Accessory breathing organs are present. They have long air sacs that extends from the gill chamber. It is capable of living outside the water for a long time due to having the air breathing organs. They are found in fresh water throughout India. They are predatory in nature but not piscivorous. Adult and juveniles mostly prefer insects, shrimps and organic debris, but larva and young fries are feeding mainly on microcrustaceans. They subsist largely on a diet of mud from the swamp bottom.

3.1.2. Equipments :-

1. Fish aquaria 45 x 30 x 25cm.
2. 20 litre pails for transfer and holding fish aquaria.
3. Fish measuring board (Metric).

4. Monopan Balance (Dhona).
5. Stainless steel forceps.
6. Stainless steel dissecting scissors.
7. Stainless steel scalpal blades.
8. Tubes for histological samples.
9. Tube racks.
10. Paper towels.
11. Plastic pool.
12. Thermostat.
13. Microscope (Olympus).
14. Micro photographic camera (Asahi pentax).
15. Microtome.
16. Aerators (Royal Mark).
17. Atomic absorption spectrophotometer (Spectr AA-20).
18. GTA-96 Graphite Tube Atomizer.
19. pH Meter.

3.1.3. Chemicals :-

1. Cadmium chloride (E.Merk).
2. Mercuric chloride (E. Merk).
3. Copper sulphate (E.Merk).
4. Potassium permanganate.
5. Metal free water.

6. Distilled water.
7. Concentrated nitric acid.
8. Concentrated sulfuric acid.
9. Perchloric acid.
11. Potassium persulphate solution.
12. Sodium chloride.
13. Hydroxylamine sulphate solution.
14. Stannous chloride.
15. Hydrochloric acid.
16. Calcium solution.
17. Lanthanum solution(Lanthanum oxide and con. HCl).
18. Hydrogen peroxide.
19. Aqua regia (3 Vol.Conc. HCl+1 Vol. Conc. HNO_3).

3.2. Method :-

In present study *C.carpio* (Linn.) were treated with Mercuric chloride and Cadmium chloride and *H. fossilis* (Bloch) were exposed to Copper sulphate solution. The methods for suggesting these experiments followed APHA (1992) and EIFAC (1983).

3.2.1. Acclimatization of fish for toxicity test :-

Live and healthy fish were collected from Yamuna river at Kalpi. Fish were checked for injury and disease and

then washed in 1% KMnO_4 solution for 5 minute. Fish were acclimatized for 15 days in laboratory condition. Collected fish were kept for quarantine for at least 7 days for parasites and disease. If more than 10% of collected fish died after second day or if they were parasitized or diseased beyond control, they were discarded. Clean and sterilized containers and equipment were used. After quarantine period the disease free fish were transported to stock tanks.

3.2.2. Experiment design :-

The test fish were exposed in at least duplicate aquarium. Each test consisted of minimum of five concentration and a control. Before treatment fish were divided in to six group, comprising 10 fishes each, placed in individual glass aquarium of 20 litre capacity and used for treatment. An untreated group of 10 fish, were maintained in separate tank, as control group.

3.2.3. Selection of the test concentrations :-

At least five metal concentration were tested. The concentration was spaced at approximately logarithmic interval. The concentration were arranged so that complete mortality occur with in a day at highest concentration used and no mortality occur in lowest concentration in the period of test.

A partial mortality in at least two intermediate concentration were obtained within the test period. The test continued for 48 hours and 96 hours. The fish were not fed during this period.

3.2.4. Biological data and observations :-

In short term tests the number of dead fish in each container were counted at least daily throughout the test. The dead organism were removed as soon as they were observed. It was more important to obtain data that defined the shape of the toxicity curve than to obtain data at pre specified time. Death is the adverse effect most often used to reflect acute toxicity.

Acute toxicity test was used to determine the harmful properties of a heavy metal. Acute toxicity test demonstrated the results within short period (4d) of exposure. A Chronic toxicity test usually continues for a relatively longer period of time often for a month or so. A chronic toxicity effect can be measured in terms of reduced growth, histological alteration and bioaccumulation of metal in gill, liver and kidney. Acute toxicity test result generally are characterized by median lethal concentration LC 50.

3.2.5. Data analysis and results :-

Statistical methods are used for analyzing data of acute and chronic toxicity tests. Acute toxicity test results are generally characterized by median lethal concentration. Lethal concentration (LC 50) is calculated by parametric procedure such as probit analysis (Finney (1981). Probit method probably is the most widely used LC 50 calculation procedure and uses the probit transformation of mortality, data in combination with a standard curve fitting technique.

This method involves manually plotting dose response data and then drawing a best fit regression line. To construct the graph, percent mortality is plotted as the ordinate against concentrations and is observed on probit paper.

3.2.6. Toxicity curve :-

It is a curve produced when, the median periods of survival of test batches of fish exposed to different concentrations are expressed in graphical form. (EIFAC 1983).

3.2.6.1. Time-response curve :-

The curve obtained by plotting the cumulative

percentage response of a test batch of fish to a single concentration of toxicant against time and a regression line is drawn to analyse the result.

3.2.6.2. Concentration response curve :-

After a given period of exposure, When the different percentage responses of batches of fish exposed to different concentrations of poison are plotted against those concentrations. A regression line is drawn with the help of analysed data.

3.2.7. Physico chemical analysis :-

The physico chemical properties of water used for the experiment are given in table 2. Water temperature varied according to the ambient laboratory conditions but average temp. (24-26°C) and a photoperiod of 12L, 12d was maintained with the help of fluorescent tube. The dissolved oxygen, pH, and hardness were measured regularly in the laboratory.

3.3. Toxicity test :-

3.3.1. Treatment of Cadmium chloride to *C.carpio* :-

C.carpio (Linn.) has been exposed to different concentration of Cadmium chloride for toxicity of metal

Cadmium on fish. There was no significant difference ($P>.05$) between mean weight of the fish used in experiments. Because metabolic activity change with size and effects the parameters to be measured individual of similar size and length were used in experiment (Canli and Furness 1993). Six aquaria, one of which was designated as control, were used to conduct the experiments. Cadmium chloride was utilized for preparation of stock solution. Five aquaria were filled with 20 L of tap water and Cadmium stock solution was added to each aquaria to make the final concentrations 3.0, 3.5, 4.0, 4.5, and 5.0 mg/L cadmium, the sixth aquaria was used as control. Ten fish were added to each aquaria and the acute effect of Cadmium concentration on mortality in 24 hours intervals was investigated. Fish were not fed during acute toxicity test. 96 hours lethal concentration of Cadmium chloride to *C. carpio* was estimated following the Trimmed Sperman Karber method. For chronic toxicity test three groups of 10 fish each were exposed separately in 3 separate aquaria (marked 240h, 480h and 720h) 20L water in each of aquaria with .32 mg/L (10% 96h LC50) Cadmium chloride solution prepared in tap water were set. Ten fish were added in each aquaria, the effect of Cadmium concentration in Gill, Liver, and Kidney were observed in 240h, 480h and 720h.

Five fish were sacrificed in different exposure duration for determination of Cadmium accumulation and histopathological abnormalities.

3.3.2. Treatment of Mercury chloride to *C.carpio* :-

Similar above experiment were conducted for determination of toxicity of Mercury on *C.carpio* (Linn.). Five aquaria were filled with 20 liter of tap water and Mercury stock solution were added to each aquaria to make the final concentration of .30, .35, .40, .50 mg/L Mercury. Ten fishes were added to each aquaria and effect of Mercury concentration on mortality were investigated in 24 hours intervals. Fish were not fed during acute toxicity test. For chronic toxicity tests three group of 10 fish were exposed in 3 separate aquaria (marked 240h, 480h and 720h) containing 20 liter tap water each with .03 mg/L (10% 96h LC 50) Mercuric chloride solution prepared in tap water. 10 fish were added in each aquarium and the effect of Mercury concentration on Gill, Liver, and Kidney was observed. Fish were sacrificed in different exposure duration for the determination of Mercury accumulation and histopathological abnormalities in Gill, Liver, and Kidney.

3.3.3. Treatment of Copper sulfate to *H. fossilis* :-

Similar above experiment were conducted for determination of toxic effect of Copper sulphate on *H. fossilis*. Five aquaria were filled with 20 liter of tap water and Copper stock solution was added to each aquarium to make final concentration to 10, 20, 30, 40, 50 mg/L Copper. Sixth aquarium was used as control. Ten fish were added to each aquarium and effect of Copper concentration on mortality were investigated in 24 hours interval. For chronic toxicity tests three group of ten fish were exposed in 3 separate aquaria marked as (240h, 480h and 720h) containing 20 L tap water each with 2.24 mg/L (10% of 96h LC 50) Copper sulphate solution. Ten fish were added to each aquaria and effect of Copper concentration on Gill, Liver and Kidney were observed. Fish were sacrificed in different exposure duration for determining Copper accumulation and histopathological abnormalities in Gill, Liver and Kidney.

3.3.4. Determination of accumulation of metal in vital organs of metal exposed fish :-

The accumulation of metal in the organs of fish has been determined by atomic absorption spectrometer. Sample containing organic matter generally require pretreatment before

analysis. Making Colorless, transparent sample, containing a turbidity < 1 NTU, odorless and only in liquid phase may be analyzed directly by Atomic Absorption Spectrometer.

Fish is dissected with clean instruments. Gill, Liver and Kidney tissue are put in digestion flask after excising from the body of fish. Preliminary digestion of metal is done by nitric acid- perchloric acid method (Approved by standard method committee 1991). Gill, Liver, and Kidney are digested separately for determination of accumulation of Copper, Mercury, and Cadmium by Atomic Absorption Spectrometer. Gill, Liver, and Kidney are separately transferred to suitable conical flasks. Sample is acidified with conc. HNO_3 and tested with methyl orange end point. 10 ml each of conc. HNO_3 and HClO_4 is added in cool flask or beaker between addition. Evaporation is done gently on a hot plate until dense white fumes of HClO_4 just appear. If solution is not clear, cover container with watch glass and keep solution just boiling until it appears clear. 10ml conc. HNO_3 is added to complete digestion solution and is then cooled and diluted to about 50 ml with water and boiled to expel any chlorine or oxides of nitrogen.

3.3.4.1. Determination of Mercury :-

Cold vapor atomic absorption method is used for determination of Mercury (APHA 1992).

3.3.4.1.1. Principle :-

Atomic absorption spectrometer resembles emission flame photometry in that a sample is aspirated in to a flame and atomized. The major difference is that in flame photometry the amount of light emitted is measured, where as in atomic absorption spectrometry a light beam is directed through the flame into a monochromator, and on to a detector that measures the amount of light absorbed by the atomized element in the flame. For some, atomic absorption spectrometer exhibits superior sensitivity over flame emission. Because each metal has its own characteristic absorption wavelength, a source lamp composed of that element is used. This makes the method relatively free from spectral or radiation interferences.

3.3.4.1.2. Apparatus :-

Glassware for Mercury analysis, Atomic Absorption Spectrometer, absorption cell, cell support, air pump, reaction flask, drying tube etc.

3.3.4.1.3. Reagents :-

A:- Metal free water :-

Metal free water is used for preparation of all the reagents and calibration standards and dilution water. Metal-free water is prepared by deionizing tap water. (NOTE: If the source water contains Hg or other volatile metals water may not be suitable for trace analysis.)

B:- Stock Mercury solution :-

.135 g Mercury chloride HgCl_2 is dissolved in about 70ml water, 1ml conc. HNO_3 is added and diluted to 100 ml with water (1.00ml=1.00 mg Mercury).

C:- Standard Mercury Solution :-

A series of standard Mercury solution is prepared containing 0 to 5 ug/L. Appropriate dilution of Stock Mercury solution is done with water containing 10 ml conc. HNO_3 /L. Standards solution is prepared daily.

D:- Nitric acid :-

HNO_3 Conc.

E:- Potassium permanganate solution :-

50 g KMnO_4 is dissolved in 1L of water.

F:- Sodium chloride-hydroxylamine sulphate solution :-

Dissolved 120g $(\text{NH}_2\text{OH})_2\text{H}_2\text{SO}_4$ in water and diluted to 1L .A 10% hydroxylamine hydrochloride may be substituted for hydroxylamine sulphate.

G:- Potassium perferate solution :-

50g $\text{K}_2\text{S}_2\text{O}_8$ is dissolved in water and diluted to 1L.

H:- Stannous Ion Sn^{+2} solution :-

10 g SnCl_2 is dissolved in water containing 20ml conc. HCl and diluted 100 ml.

I:- Sulfuric acid :-

H_2SO_4 conc.

3.3.4.1.4. Procedure :-**A:- Instrument operation :-**

Set wave length to 253.7 nm. Install absorption cell and align in light path to give maximum transmission. Connect associated equipment to absorption cell with glass tubing. Turn on air and adjust flow rate to 2 L/min. Allow air flow continuously.

B:- Standardization :-

100 ml of each 1.0, 2.0, 5.0 $\mu\text{g/L}$ mercury solution and blank of 100ml water is transferred to 250ml reaction flasks. Add 5 ml conc. H_2SO_4 and 2.5 ml conc. HNO_3 to each flasks. Add 15 ml KMnO_4 solution to each flask and let that stand for at least 15 min. Add 8 ml $\text{K}_2\text{S}_2\text{O}_8$ solution to each flask and heat for 2 hours in a water bath at 95°C , cool to room temperature.

Each flask is treated individually with enough NaCl . Hydroxyl amine sulphate solution is added to reduce excess KMnO_4 and then 5ml SnCl_2 solution is added and immediately flask is attached to aeration apparatus. Mercury was volatilized and carried to the absorption cell. Absorbance is increased maximum for few seconds. As soon as recorder returns approximately to base line stopper is removed holding the frit from reaction flask, and replace with a flask containing water. Flush system for few seconds and run standard curve by plotting peak highest versus micrograms Mercury.

3.3.4.1.5 Calculation :-

Determine peak height of sample from recorder chart and read Mercury value from standard curve preparation.

3.3.4.2. Determination of Cadmium and Copper :-

Direct air-acetylene flame method is used for determination of Copper and Cadmium (APHA 1992).

3.3.4.2.1. Principle :-

Principle is same as that of determination of Mercury.

3.3.4.2.2. Apparatus :-

Atomic absorption spectrometer and associated equipment.

3.3.4.2.3. Reagents :-**A:- Air :-**

Clean and dried through a suitable filter to remove, oil, water, and other foreign substances.

B:- Acetylene :-

Standard commercial grade.

C:- Metal free water :-

Use metal free water for preparing all reagents and for dilution in water. Prepare metal free water by deionizing tap water. (NOTE: If the source water contains Hg or volatile metals, single or redistilled water may or may not be suitable for trace analysis because these metals distil over).

D:- Calcium solution :-

Dissolved 630 mg calcium carbonate (CaCO_3) in 50ml of 1+5 HCl. If necessary, boil gently to obtain complete solution. Cool and dilute to 1000ml with water.

E:- Hydrochloric acid :-

HCl 1%, 10%, 20%, 1+5, and Conc.

F:- Lanthanum solution :-

Dissolved 58.65g lanthanum oxide La_2O_3 in 250 ml conc. HCl. Add acid slowly until the material is dissolved and dilute to 1000ml with water.

G:- Hydrogen peroxide :-

30%

H:- Nitric acid :-

HNO_3 2%, 1+1 and conc.

I:- Aqua regia :-

Add 3 volumes conc. HCl to 1 volume conc. HNO_3 .

J:- Standard metal solution :-

Prepare a series of standard metal solution in the optimum concentration range by appropriate dilution of following stock metal solution with water containing 1.5ml conc HNO_3 /L.

[I]:- Cadmium :-

Dissolve .100gm Cadmium metal in 4ml conc. HNO_3 .

Add 8ml conc. HNO_3 and dilute to 1000ml with water;

1.00ml=100 μg Cd.

[II]:- Copper :-

Dissolve .100g Copper in 2ml conc. HNO_3 and dilute

to 1000ml with water; 1.00ml=100 μg Cu.

3.3.4.2.4. Procedure :-

A. Instrument operation :-

Install a hollow-cathode lamp for the desired metal in instrument and approximate wave length is set. Set the width according to manufacture's suggested setting for element being measured. Turn on instrument, apply hollow-cathode lamp to the current suggested by the manufacture, and let instrument warm up until energy source, stabilizes, generally about 10 to 20min.. Read the current just necessary after warm up. Optimum wave-length is maintained by adjusting wavelength dial until optimum energy gain obtained.

Install suitable burner head and adjust burner head position. Turn on air and adjust flow rate to that specified by manufacturer to give maximum sensitivity for metal being measured. Turn on acetylene, adjust flow rate to value specified and ignite flame. Let flame stabilize for a few minutes. Aspirate a blank consisting of either deionized water or an acid solution containing the same concentration of acid in standards and sample. Zero the instrument. Aspirate a standard solution and adjust aspiration rate of the nebulizer to obtain maximum sensitivity. Adjust burner both vertically and horizontally to obtain maximum response. Aspirate blank again and re-zero

the instrument. Aspirate a standard near the middle of linear range. Record absorbance of this standard when freshly prepared with a new hollow cathode lamp. Refer to these data on subsequent determination of same element to check consistency of instrument setup and aging of hollow cathode lamp and standard.

The instrument now is ready to operate. When analyses are finished, extinguish flame by turning off first acetylene and then air.

B. Standardization :-

Select at least three concentration of each standard metal solution. Aspirate blank and zero the instrument. Then aspirate each standard in turn to flame and record absorbance.

Prepare a calibration curve by plotting on linear graph paper the absorbance of standards versus their concentration.

C. Analysis of sample :-

Rinse nebulizer by aspirating water containing 1.5 ml conc. HNO_3 /L. Atomize blank and zero instrument. Atomize sample and determine its absorbance.

3.3.4.2.5. Calculation :-

Calculate concentration of each metal ion, in microgram per liter for trace elements, by referring to appropriate calibration curve prepared according to standardization. Alternatively, read concentration directly from the instrument. Read out if the instrument is so equipped.

3.3.5. Histopathology :-

Histopathological studies were used to determine pathological alteration in the structure of tissue. In this investigation fish *C. carpio* has been treated with Mercury and Cadmium and *H. fossilis* treated with the Copper for different periods. Due to metallic treatment, some abnormality appeared in selected organs Gill, Liver and Kidney of fish.

3.3.5.1. Sample collection for Gill histology :-

The Gill tissue begin to degrade almost immediately after death and must be removed as quickly as possible. After opening the opercula second Gill arch on both sides of fish is selected. The second gill arches are cut with a pair of scissors taking care to cut it from the base to dorsal and ventral insertion. The gill arches are placed in a labeled sample tube and filled with 10% neutral buffered formalin.

3.3.5.2 Sample collection for liver histology :-

Make longitudinal section through the middle of the liver and remove a strip of tissue. Place the sample in to sample tube and preserved in 10% neutral buffered formalin. For histology sample, the liver tissue should originate from a portion of liver away from bill duct.

3.3.5.3. Sample collection for kidney histology :-

Preserve the kidney in sample tube in 10% neutral buffered formalin.

3.3.5.4. Staining Technique :-

Haematoxylin(nuclear stain) and eosin (cytoplasmic stain) were used for double staining process for histological studies.

A. Ehrlich's acid Haematoxylin :-

A:-	Haematoxylin	2gm
B:-	Absolute alcohol	100ml
C:-	Glycerine	100ml
D:-	Glacial acetic acid	10ml
E:-	Distilled water	100ml
F:-	Alum in excess	

Haematoxylin is dissolved in absolute alcohol, acetic acid is added and then the glycerine and water is mixed. The mixture is allowed to ripen in the light until it acquires a dark red colour.

B. Eosin :- A:- Eosin powder 1.0gm

B:- 90% alcohol 100ml

Dissolved 1 g of eosin in 100 ml of ethyl alcohol.

The slide is dipped in xylol .In this step individual slide require two changes in xylol (15 minutes in the first and 5 minutes in the second). The wax is completely dissolved and removed and only section material remains on the slide. Now pass the slide in degrading series of alcohol. Absolute, 90%, 70%, 50%, 30% distilled water. Fine result are obtained by keeping the slide in these series for 8-10 minutes. Two change in distilled water are required each of 5 minutes. Then stain the section in aqueous Haematoxylin for 2-5 minute. After staining dip the slide in distilled water. Then dehydrate the section on the slide through ascending series of alcohol 30%, 50%, 70% and 90%. Then stain the section in alcoholic eosin. Then the slide is kept in a staining trough containing absolute alcohol. Add xylol for 5 minute and then finally the slide is transferred in the pure xylol for 15 minute. Then the slide is mounted with Canada balsum.

CHAPTER -4

**OBSERVATIONS AND
RESULTS**

4.1 Acute toxicity test:-

Acute toxicity test is useful for the determination of harmful properties of metal. Acute toxicity test is demonstrated with in a short period of exposure. Exposure period for these test usually is 48h or 96h. Death is the adverse effect, which is most often used to reflect acute toxicity. Death is an important and easily detectable adverse effect. The usual criterion for death is, no movement in the fish, especially no gill movement and no reaction to gentle prodding. Acute toxicity test results are generally characterized by Median lethal concentration (LC 50).

4.1.1 Acute toxicity test of Cadmium chloride in

C. carpio :-

Fish *C. carpio* (linn.) have been subjected to different concentrations of Cadmium chloride to study the effect of its toxicity. In the five aquaria filled with 20L of tap water, Cadmium stock solution were added to each aquaria to make final concentration 3.0, 3.5, 4.0, 4.5, and 5.0 mg/L. Sixth aquaria was maintained as control condition (without Cadmium chloride). Ten fishes were added in each aquaria. The effect of Cadmium concentrations on the mortality of fish was investigated after

24h interval. The percentage of mortality were summarized in (table 3) and displayed in fig-1. The acute toxicity test were characterized in terms of median lethal concentration. The median lethal concentration at 24h, 48h, 72h, and 96h, were estimated by probit analysis (APHA 1992 and Finney 1981) and represented in fig-2, 3, 4, and 5. The values of LC 50 at 24h, 48h, 72h and 96h are summarized in table 5. Fig-6 indicated that LC 50 values decrease with increased test duration. Percentage of mortality were observed at 0, 20, 30, 40 and 50% respectively at each period of 24h, 48h, 72h, 96h and 120h in an aquaria having 3.0mg/L concentration of Cadmium chloride. In aquaria having concentration 5.0mg/L showed 90% mortality at 120h. The data thus obtained were subjected to standard statistical processing, regression analysis. Regression line in fig 19, 20, 21, 22, and 23 displays relationship between exposure duration and percentage of mortality of *C.carpio*. High positive value of correlation coefficient (r) obtained, represent a strong correlation between exposure duration and percentage of mortality at each constant concentrations.

Mortality is also affected by concentration of metal. Regression line in fig-25, 26, 27, and 28 is showing correlation between concentration of metal and percentage of mortality of

fishes. Therefore, by drawing a regression line any value of percentage of killed fishes can be predicted at any concentration of Cadmium chloride within natural occurring limits. High positive value of correlation coefficient (r) in fig 25, 26, 27, and 28, indicated strong correlation between concentration of metal and percentage of mortality in constant exposure duration. The value of r^2 (.98) in fig-26 shows that 98% of variation in percentage of killing are influenced by concentration of metal in constant exposure duration. However remaining 2% variation may be attributed to other factors such as temperature and pH. It is established from above observations that percentage of mortality is dependent on concentration of metal as well as exposure duration.

4.1.2 Acute toxicity test of Mercuric chloride in *C. carpio*:-

The toxic effect of Mercuric chloride on *C. carpio* (Linn.) has been determined by acute toxicity. Five aquaria filled with 20 L of water and Mercury stock solution was added to each aquaria to make a final concentration .30, .35, .40, .45, and .50 mg/L. Sixth aquaria maintained as control condition (without Mercuric chloride). The mortality was calculated as

percentage once every 24 h. The percentage of mortality in *C. carpio* due to different concentration of Mercuric chloride in different duration have been summarized in table-4 and represented in fig-7.

Percentage of mortality have been noticed 20, 30, 40, 60, and 70% respectively in .30, .35, .40, .45, and .50 mg/L concentration at 48 h of exposure duration. In .30mg/L concentration 10% mortality of *C. carpio* has been noticed at 24h. All the fishes were killed at 120h exposure in aquaria having .50mg/L concentration. The value of LC 50 at different hours 24h, 48h, 72h and 96h were estimated by probit analysis (fig- 8, 9, 10, and 11) and summarized in table 6. Fig 12 indicated that value of LC 50 decreased with increased time duration. Regression line and high positive value of correlation coefficient (r) in fig 29, 30, 31, 32, and 33, displaying relationship between exposure duration and percentage of mortality in constant concentration of Mercuric chloride, in *C. carpio* have been observed. Regression line in fig (35, 36, 37, and 38) indicates co-relation between concentration and percentage of mortality in constant duration. It is cleared that percentage of mortality is influenced by concentration as well as exposure duration.

4.1.3 Acute toxicity test of Copper sulphate in *H. fossilis* :-

The toxic effect of copper sulphate in *H. fossilis* (bloch) have been investigated by acute toxicity tests. Five aquaria filled with 20L of tap water and Copper stock solution were added to each aquaria to make final concentration of 10, 20, 30, 40, and 50mg/L. Sixth aquaria was maintained as control condition (without Copper sulphate). Ten fishes were added in each aquaria. The mortality was calculated once at 24h intervals. All experiment were run for 120h.

Percentage of mortality in *H. fossilis* due to different concentration of Copper sulphate in different exposure duration were summarized in table 7 and represented in fig-13. In 20 mg/L concentration 10% mortality were noticed at 24h. All the fishes were killed at 120h in aquaria having 50 mg/L. LC 50 values at different hours 24h, 48h, 72h, and 96h were estimated by probit analysis represented in fig-14, 15, 16, and 17 and summarized in table 8. Fig-18 indicates that LC 50 value decreases with increasing the test duration. Regression line in fig 39, 40, 41, 42, and 43 shows strong correlation between exposure duration and percentage of mortality in constant concentration of Copper sulphate. High positive value of

correlation coefficient and regression line in fig-45, 46, 47, and 48 indicates strong correlation between concentration and percentage of mortality in constant exposure duration. It is evident from the above observations that percentage of mortality is affected by concentration as well as exposure duration of metal.

4.2. Behaviour abnormalities :-

Behaviour profile showed large difference between *H. fossilis* and *C. carpio* in swimming movement, feeding and social behaviours. *C. carpio* and *H. fossilis* were exposed to sub-lethal concentration of Copper sulphate, Mercuric chloride and Cadmium chloride. Certain changes were observed in swimming pattern, feeding behaviour and activeness. Both the fishes initially became more active but later their activity ceases. Both types of exposed fishes showed fading in coloration a little and fluctuation responses were observed in feeding behaviour. Exposed fishes also showed increase in ventilation movement of operculum and increased gulping activity. Secretion of excessive mucus has been observed all over the body surface of exposed fishes. More amount of mucus were latter released into media at various stages. Exposed fishes reject food especially in the early stages of exposure. Throughout the duration of the experiment following changes were compared to the control group.

4.2.1 Alteration of behaviour in *C.carpio* exposed to Cadmium chloride :-

In present study in different concentration of Cadmium chloride i.e. .32 mg/L, 1.5 mg/L, 3.0 mg/L with exposure duration for 1 week, alterations of behaviour in *C.carpio* have been observed. Fishes showed normal active swimming pattern in control condition (without Cadmium chloride). The lowest treatment of Cadmium chloride (.32 mg/L) caused little change in behaviour such as locomotary activity. Significant response to 1.5 mg/L concentration of cadmium chloride was noticed. The opercular movement and swimming activity were increased and lethargic response was observed in prolong time of exposure. In high concentration of 3.0 mg/L, in early period of exposure the fish showed lethargic response and later *C. carpio* showed loss of equilibrium . The observation has been summarized in table-9.

4.2.2. Alteration of behaviour in *C.carpio* exposed to Mercuric chloride:-

At the low treatment levels, fish showed significant effect over time. The change of behaviour in *C.carpio* (Linn.) in different concentration of Mercuric chloride respectively in

.038 mg/L, .10 mg/L and .15 mg/L have been observed. Fishes exposed for 1 week to very low concentration of Mercuric chloride showed little response in earlier period and later fish showed increase in swimming activity and opercular movement in comparison to control condition. Mercury is highly toxic as compared to Cadmium so fish showed high toxic stress for Mercury than Cadmium. In .10 mg/L concentration fish showed increase in opercular movement and lethargy. Fish became sluggish (more amount of mucus released in media) and loss of equilibrium has been noticed in *C.carpio* exposed to high concentration of mercuric chloride (.15 mg/L) for 1 week. The observation have been summarized in table-10.

4.2.3. Alteration of behaviour in *H.fossilis* exposed to Copper sulphate:-

In normal control condition (without Copper sulphate) *H.fossilis* (bloch) showed normal active swimming pattern in aquarium and remained at one corner of aquarium. The alteration in behaviour of *H.fossilis* in different concentration of Copper sulphate respectively in 2.24 mg/L, 5.0 mg/L, and 10 mg/L for exposure duration of 1 week has been observed. The lower concentration of Copper sulphate 2.24 mg/L caused

little change in behaviour pattern such as increase in opercular movement and air gulping activity. In earlier stage of exposure, fish showed jerky and erratic swimming movement. In 5.0 mg/L concentration of Copper sulphate it induced swimming activity, lethargic response, frequent surfacing along with gulping of air. Exposed fishes showed secretion of excessive mucus all over the body. Significant response appeared in 10mg/L Copper sulphate concentration. In this concentration fish showed lethargy and loss of equilibrium and fish became restless and sluggish. The observation has been summarized in table-11.

4.3. Growth Abnormalities :-

4.3.1. Effect on the growth rate of fish *C.carpio* due to Cadmium chloride treatment:-

Fish *C.carpio* (Linn.) have been subjected to different concentration of Cadmium chloride to study the effect of Cadmium chloride on growth of fish. Five aquaria filled with 20L tap water and Cadmium stock solution was added to each aquaria to make a final concentration of .5, 1.0, 1.5, 2.0, 2.5, mg/L. Sixth aquaria were maintained as control condition (no Cadmium chloride). Five fish were added to each aquaria.

All the experiments were run for 20 days. The growth performance in *C. carpio* was noticed after 20 days in different concentration of Cadmium chloride. Data has been summarized in table-15. The graph has been plotted using the data of the table 15. The graph no. 49 indicated that control group of fish showed high growth rate of 9.27%. When fish were treated with .5mg/L concentration of Cadmium chloride for 20 days, the fish gained 6.90% weight. The gained weight of 6.60% was observed in aquaria having 1.0 mg/L concentration of Cadmium chloride. The 3.75% weight gain was recorded in 1.5 mg/L concentration of metal. In 2.0 and 2.5 mg/L concentration of Cadmium chloride fish lost weight by 1.35 and 1.58% respectively. It was noticed that fish growth rate reduced by the increase in concentration of metal.

4.3.2. Effect on growth rate of the fish *C. carpio* due to Mercuric chloride treatment:-

Fish *C. carpio* (Linn.) have been subjected to different concentration of Mercuric chloride to study the effect of Mercury on growth. Five aquaria were filled with 20L of water and Mercury stock solution was added to each aquaria to make a final concentration of .10, .15, .20, .25, .30 mg/L. Sixth aquaria was maintained as control (without Mercuric chloride). Five fish were added to each aquaria. All experiments were run for 20 days.

The observation of experiment has been represented in table-16. The graph no. 50 has been plotted by using the data of table-16. When fish were treated with .1 mg/L concentration of Mercuric chloride fish gained the weight by 6.48%. The fish weight gain of 5.60 and 4.48% has been observed in concentration .15 and .20mg/L respectively. In high concentration of .25 mg/L the fish lost 1.71% weight. In controlled condition (without Mercuric chloride) fish gained high percentage weight (10.48 %).

4.3.3. Effect on growth rate of the fish *H.fossilis* due to Copper sulphate treatment:-

The *H.fossilis* (bloch) has been treated to different concentration of Copper sulphate to study the effect of metal on growth of fish. Five aquaria were filled with 20L of water and stock solution of metal was added to each aquaria to make a final concentration of 1, 5, 10, 15, 20 mg/L. Sixth aquaria were maintained as control (without Copper sulphate). Five fish were added to each aquaria. All experiments were run for 20 days. The observation of experiment was recorded in table-17. The graph no. 51 have been plotted by using the data of table-17. When the fish were treated with 1.0 mg/L concentration of

Copper sulphate for the duration of 20 days, fish gained weight by 5.56%. The 4.84% and 2.52% weight was gained by fish in concentration of 5 and 10mg/L Copper sulphates. In higher concentration of 15 and 20 mg/L, the fish lost weight by 1.62 and 2.56%. It was noticed that fish growth rate got reduced by increasing the concentration of Copper sulphate.

4.4. Histological changes in fish due to heavy metal:-

The present study was carried out to observe the effect of metal on the histology of vital organs of the fish. For the study of histological alterations due to the effect of metal, *C. carpio* (Linn.) has been treated with Cadmium chloride and Mercuric chloride. *H. fossilis* (Bloch) was exposed to Copper sulphate. Gill, liver and kidney sample were fixed in Bouins fixative for 8 hours, then transferred for dehydration in increasing ethanol series and embedded in paraffin. At least two slide with 4, 5 section of each sample were examined under Olympus microscope.

4.4.1. Gill histology:-

The gills are the primary respiratory organ of fish. The gill epithelium of fish is a major site of gas exchange, acid base balance regulation and excretion of nitrogenous waste. Gill arch is composed of numerous gill filaments with two rows of

secondary lamellae that run perpendicular to each filament. Secondary gill lamellae are composed of single layer of epithelial cells and supported by pillar cells. Chloride cells were identified as large epithelial cell with light cytoplasm, usually present in the base of the lamellae. Mucus cells were present in epithelium of the filament at the base of lamellae (P-3).

4.4.1.1. Histological changes in the gill of *C. carpio* due to Cadmium chloride:-

Fish *C. carpio* (linn.) were treated with different concentration of Cadmium chloride and different exposure duration to study the alteration of histology. After 96 hours exposure to .32mg/L Cadmium chloride fish gill showed primary epithelial lifting, thickening of primary lamella and mucus cells (P-4). When fish were exposed for 20 days in .32 mg/L concentration, fish gill showed hyperplasia and hypertrophy of chloride cells and mucus cell at the base of gill filament due to which thickening of primary lamella and fusion of secondary lamellae has been observed (P-5). At high concentration 1.0 mg/L Cadmium chloride for 30 days the lamellae were observed to be thin and elongated. The pillar cells appear with much reduced size. Rapid lysis in the epithelial cells with complete disruption of these cells have been observed (P-6).

4.4.1.2. Histological changes in the gill of *C. carpio* due to Mercuric chloride:-

Fish *C. carpio* (linn.) were exposed to .03 mg/L concentration of Mercuric chloride and different exposure duration for the investigation of the alteration of histological abnormalities. After 96 hours fish gill showed thickening of primary lamellar epithelium and epithelial lifting (P-7). Fish exposed to .03 mg/L Mercuric chloride for 20 days, gill showed aneurism (P-8) of epithelial cells and fusion of lamellae. At the same concentration and prolong exposure duration (30 days) fusion of two or more lamellae restricting the passing of water is observed (P-9).

4.4.1.3. Histological changes in the gill of *H. fossilis* due to Copper sulphate:-

The live fish *H. fossilis* (bloch), an important edible cat fish has bimodal breathing because it can respire aurally by gulping in air at various intervals when oxygen content of water is reduced below saturation point. Fish *H. fossilis* has been exposed to 2.24 mg/L (10% of 96h LC 50) concentration of Copper for different exposure duration. Five experimental fish and five control fish were sacrificed by cervical dislocation after expiry of

96 hours, 10 days, 20 days, of exposure. Control group of fish *H.fossilis* showed normal appearance of primary lamellar epithelium, chloride cell, epithelial cell, mucus cells and pillar cells (P-10). After 96 hours of exposure fish gill showed epithelial lifting, thickening of primary lamellar epithelium and mucus cells (P-11). At the same concentration for prolonged duration of 20 days fish gill showed thickenings of epithelium at the tip of lamella (telangiectasis) (P-12). Fish gill showed tumor (P-13) when were fish exposed to 2.24 mg/L Copper sulphate for 30 days.

4.4.2. Liver histology:-

Liver plays a major role in metabolic activities of excretion, digestion and storage of various substances including some, which are toxic to fish. The liver of fish is the largest extrinsic digestive gland and a site of initial processing of materials absorbed by intestinal capillary and transferred via hepatic portal vein. Toxic material absorbed with foodstuffs can enter the liver and affect its structure and function. The liver of fish is typically parenchymatous. The parenchyma cells are arranged in a lattice network.

4.4.2.1. Histological changes in liver of *C. carpio* due to Cadmium chloride:-

Fish *C. carpio* (linn.) were exposed to .32 mg/L Cadmium chloride for different exposure duration for the investigation of the histological abnormality. Control Group (without Cadmium chloride) showed normal hepatocyte with spherical nuclei (P-14). After 20 days exposure to .32 mg/L Cadmium chloride fish liver showed several degenerative changes in hepatocyte. Progressive increase of fibrous connective tissue, and abnormal growth of cell were noticed (P-15). At prolonged exposure of 30 days (P-16) liver area showed focal necrosis.

4.4.2.2. Histological changes in liver of *C. carpio* due to Mercuric chloride:-

The liver of the fish exposed to .03 mg/L Mercuric chloride showed several pathological changes. After 96 hours of treatment fish liver displayed growing necrosis and several inflammation (P-17). In .03 mg/L Mercuric chloride, 20 days of exposed fish liver showed irregular clumping and tumor (P-18). In prolong exposure and same concentration liver showed necrosis and increase in surface area of liver (P-19).

4.4.2.3. Histological changes in liver of *H.fossilis* due to Copper sulphate:-

The liver of untreated fish showing the parenchyma cell arrange to form lattice network. The inter spaces are sinusoid of thin strip with sparse connective tissue (P-20). At the 20 days of exposure, irregular clumping were noticed in liver of *H.fossilis* (P-21) in 2.24 mg/L concentration of Cadmium chloride. At 30 days interval liver shows lyses of cells (P-22) and the density of connective tissue increased.

4.4.3. Kidney histology:-

Kidney are paired longitudinal structures that lie above the body cavity, ventral to the vertebral column. Redish, brown, pulpy, and bloody when broken. Histologically, the kidney is made up of nephrons, each consisting of glomerulus and tubes. The inter tubular space is filled with lymphoid tissue which is unevenly distributed.

4.4.3.1. Histological changes in kidney of *C.carpio* due to Cadmium chloride:-

No recognizable change was noticed in kidney of control fish. The kidney was composed of numerous renal

corpuscles with well developed glomeruli and system of tubules. The proximal segment is covered by columnar epithelium with basal nuclei (P-23). *C. carpio* was exposed to .32 mg/L concentration of Cadmium chloride for different exposure duration to investigate histological abnormalities of kidney. The kidney of fish exposed to .32 mg/L Cadmium chloride for 20 days showed glomerular alteration and presence of large lipid vacuole (P-24). At 30 days interval and same concentration of Cadmium chloride the kidney showed swollen epithelial cells and glomerular deterioration and presence of large lipid vacuole (P-25).

4.4.3.2. Histological changes in kidney of *C. carpio* due to Mercuric chloride:-

The fish *C. carpio* (Linn.) was exposed to .03 mg/L Mercuric chloride in different exposure duration to study the histological alteration in kidney. After 96 hours marked abnormal changes in kidney such as glomerular distortion and swollen epithelial cells (P-26) were noticed. In prolonged exposure duration for 20 days and .03 mg/L concentration of Mercuric chloride, swelling in epithelial cells, glomerular distortion and necrosis (P-27) were observed. 30 days of exposure showed

necrosis and pycnosis of epithelial cells of the tubules and glomerular distortion (P-28).

4.4.3.3. Histological changes in kidney of *H.fossilis* due to Copper sulphate:-

Kidney tissue of the control fish *H. fossilis (bloch)* showed a normal appearance of glomeruli, proximal tubule and nucleus (P-29). In 2.24 mg/L concentration after 20 day of exposure fish kidney showed hypertrophy in epithelial cell and necrosis (P-30). After 30 days treatment kidney showed abnormal appearance of glomeruli and proximal tubule (P-31).

4.5. Bioaccumulation of metal in selected tissue of fish:-

4.5.1. Bioaccumulation of Cadmium in *C.carpio* :-

C. carpio (Linn.) has been treated to Cadmium chloride for different durations to investigate the accumulation of metal in gill, liver and Kidney. Four groups of 10 fishes each were exposed separately in four aquaria with 20L water (marked as 240h, 480h, and 740h) containing each of .32 mg/L (10% of 96h LC 50) Cadmium chloride prepared in tap water. Parallel group of 10 fishes were kept in separate aquaria containing 20 L of tap water (without the addition of Cadmium chloride) as control.

After the expiry of 240h, 480h and 720h of exposure 3 fish each from the respective marked experimental aquaria as well as control aquarium, were sacrificed. For estimating the Cadmium content, gill, liver and kidney were excised from experimental as well as control fish separately and tissue were placed in petri dishes and digested according to APHA standard method. Metal concentration in sample were measured using atomic absorption spectrophotometer. Estimated data are presented in (Table -12).

4.5.1.1. Gill:-

In all the tissues observation, the rate of accumulation of Cadmium has been recorded maximum in the gills of exposed fish and no detectable amount of Cadmium was observed in control fish. The mean accumulation of Cadmium after 30 day of exposure was $.051\mu\text{g/g}$ (Fig-52).

4.5.1.2. Liver:-

The accumulation of Cadmium was less in the case of liver as compared to gill. The pattern of accumulation shows more or less continuous increasing trend except for 720h of exposure (Table-12). The mean rate of accumulation was $.038\mu\text{g/g}$ (Fig-52).

4.5.1.3. Kidney:-

The rate of accumulation of cadmium in kidney increased with the exposure time. The mean accumulation of cadmium in kidney during the sub lethal exposure was .035 µg/g which is next to that of liver (Fig-52). Cadmium was not detected in control.

4.5.2. Bioaccumulation of Mercury in *C.carpio*:-

The *C.carpio* (linn.) has been exposed to Mercuric chloride to study the accumulation of Mercury in tissue of fish. Five aquaria were filled with 20L of water. Four group of 10 fish each were exposed separately in four aquaria (marked 240h, 480h, and 720h and a control) containing 20L water .03 mg/L (10% of 96h LC 50) Mercuric chloride prepared in tap water. Parallel group of 10 fishes kept in separate aquaria containing 20 L of tap water (without the addition of Mercuric chloride). After the expiry of 240h, 480h, and 740h of exposure, 3fish each from the respectively marked experimental aquaria as well as control aquaria were sacrificed. For estimation of the mercury accumulation in fish, a pair of gill, liver and kidney were excised from experimental fish as well as control fish and placed in petry

dishes and digested. Metal concentration in sample were measured using atomic absorption spectrometer. Obtained data has been tabulated in (Table 13).

4.5.2.1. Gill:-

The accumulation of mercury was less in the case of gill when compared to liver. The pattern of accumulation shows continuously increasing trend. The mean accumulation of Mercury in gill during the sub lethal exposure was $.0047\mu\text{g/g}$ (Fig-53).

4.5.2.2. Liver:-

The accumulation of mercury in liver increased along with exposure time. The mean accumulation of Mercury in liver during the sub lethal exposure was $.0063\mu\text{g/g}$ (Fig-53) which is highest as compared to gill and kidney.

4.5.2.3. Kidney:-

In all the tissues estimation, the rate of accumulation of Mercury was observed minimum in kidney of exposed fish, and no detectable amount of Mercury was observed in the kidney of control fish. The mean accumulation of Mercury in kidney after 30 days of exposure was $.0037\mu\text{g/g}$ (Fig-53).

4.5.3. Bioaccumulation of Copper in *H.fossilis*

Fish *H.fossilis* (bloch) were exposed to 2.24 mg/L of Copper sulphate and different exposure duration to study the accumulation of copper in tissue of fish. Four group of ten fish each were exposed separately in four aquaria (marked as 240h, 480h and 720h) containing 20 L of water and 2.24 mg/L (10% of 96h LC 50) copper prepared in tap water. The forth aquaria was kept as control (without copper sulphate). After expiry of 240h, 480h, and 720h of exposure, 3 fish from the respectively marked aquaria as well as control aquaria were sacrificed. For estimating Copper content gill, liver and kidney were excised from experimental fish and control fish separately and tissue were placed in petry dishes and digested. Metal concentration in sample was estimated using atomic absorption spectrophotometer. Obtained data has been tabulated in (Table-13).

4.5.3.1. Gill

The rate of accumulation of copper in gill was found to be increase with increasing exposure time. The accumulation of copper was less in the case of gill as compared to liver. The

mean accumulation of copper in gill after 30 days of exposure was $2.99 \mu\text{g/g}$ (Fig-54).

4.5.3.2. Liver:-

In all tissue observation, the rate of accumulation of copper was maximum in Liver of exposed fish and the mean accumulation of Copper in liver after 30 days of exposure was $32.01 \mu\text{g/g}$ (Fig-54).

4.5.3.3. Kidney:-

It was observed that accumulation of Copper in kidney, increased along with exposure time. The mean accumulation of copper in kidney during sub lethal exposure was observed as $2.09 \mu\text{g/g}$ (Fig-54), which is next to the gill. Copper was not detectable in the control fish.

CHAPTER -5

DISCUSSION

5.0 General :

Heavy metals effect the fish in different ways such as alteration of behaviour pattern, growth and histology of different organs. Bioaccumulation of metal in different organs of animals have been observed in many studies. When fish was exposed to different concentration of metal in an aquaria, they tend to take these metal from their direct environment. It is assumed that metals are taken up in the ionic form and that this uptake is influenced by various environmental factors such as pH, hardness and temperature (Cusimano et. al. 1985). LC 50 values has been observed to increase in fish with hardness of water (Kallanagoudar and Patil 1997). This would mean that toxicity of metal remains lower in hard water. Contrary to hardness reduction in water pH favored accumulation of Hg in *Gambusia* (Paulas 2004). Rise in the temperature of water also increases the toxicity of metal (Khangarot et. al. 1981b).

Nutritionally heavy metals are antagonistic to essential trace element in properties. Heavy metals compete with nutrient elements from their binding site of transporter protein and metalloenzyme receptors. Metals disrupt the metabolic balance of nutrient elements due to metabolic disruption of

carbohydrate, protein and amino acid. Some observation have showed that heavy metals could decrease the glycogen reserve in the fish (Levesque et. al. 2002) by affecting activity of enzyme that play a role in carbohydrate metabolism.

The metal enter the body of fish via gill and skin through intake of contaminated water. Transport of metal occurs in the fish through the blood where ions are usually bound to protein. The metals are thus brought into contact with organs and tissue of fish and accumulate in different organ and tissue.

In the present study *C. carpio* (Linn.) has been exposed to the sub lethal concentration of Cadmium and Mercury salts and *H. fossilis* (Bloch) is exposed to sub lethal concentration of Copper salt. In this chapter an attempt has been made to correlate results of different parameters such as behaviour and growth alteration due to the effect of metal. Heavy metals have also affected the histology of organs due to their accumulation in vital organs.

5.1 Acute Toxicity of Metal on Fish:-

The LC 50 test are conducted to measure the susceptibility and survival potential of an organism to particular

toxic substance such as heavy metal. Higher LC 50 value represent less toxicity because greater concentration is required to produce 50% mortality in organism (APHA 1992). LC 50 values of fish varies from fish to fish and metal to metal.

Sphera et. al. (1982) reported 96h LC 50 values to be 2.5 and 28.0 ppm of Cd for *Jordanella floridae* and *Mugil cephalus* respectively. Das and Banerjee (1980) found the LC 50 value to be 175.0 and 300.0 ppm Cd for *H.fossilis* and *Labeo rohita* respectively. Shastry and Gupta (1994) found 96h LC 50 value to be 13.2ppm of Cd for *Channa punctatus*. Panchanathan and Issac (2006) reported that 96 h LC 50 value were 70ppm of Cd for *Clarius batrachus*. Smelt and Blust (2001) observed 100% mortality in *C. carpio* after 29 days of exposure to 20mg Cd. Hwang et. al. (1995) reported 96 h LC 50 value to be .029 mg/L for Cadmium in 9 day old *Oreochromis mossambicus*. Khangarot (1981a) and Das et. al. (1980) reported 96h LC 50 values of .312, .35 and .145 ppm of Mercury for *Channa marulius*, *Heteropneustes fossilis*, *P. sophore* respectively. Chen and Yang (2007) have investigated the acute toxicity of the antimony and determined 14.05 mg/L as 96h LC 50 value for antimony to *Cyprinus carpio*.

Paulose (1987) has found 96 h LC 50 values 230ppb and 180ppb Mercury for *Gambusia affinis* and *Labeo rohita* respectively. Bhoopathy et. al. (2000) have observed 24h LC 50 value of .54 ppm Hg for *O. mossambicus*. Gill and Pant (1985), Kirubakaran and Joy (1988), Veena et. al. (1997) and Iliopoulou-Georgudaki (2001) have reported 96 h LC 50 values of .181, .51, .13, and .51 ppm Mercury for *Barbus conchoni*, *Clarius batrachus*, *Etrophus maculatus* and *Salmo gairdneri* respectively.

Copper is an essential metal for living being. Bhatia (1970) has reported 96 h LC 50 value .10 and 1.0 ppm of Copper for *Cirrhinus mrigala* and *Colisa fasciatus* respectively. Radhakrishnaiah (1988) have found LC 50 96h to be 1.2 ppm Copper in *Labeo rohita*. Khangarot and Ray (1987) found 96h LC 50 .986ppm of Copper at 28 °C for *Poecili reticulata*. The LC50 values of Copper at 24 h and 96 h have been reported to be 19 and 18.5 ppm respectively in *Puntius ticto* by Mishra and Sulochana (1995). Griffith et. al. (2007) reported that soluble Copper sulphate was highly toxic to female Zebra fish, with 48h static LC 50 of .25mg Cu/L. They have also examined the acute toxicity to be 80nm of Copper nanoparticle suspension on Zebra fish. They have demonstrated that nanocopper is acutely toxic

to zebra fish (*Danio rerio*), with 48h LC50 concentration of 1.5 mg/L (nanoparticle suspension).

In the present study the 96 h LC 50 in *C. carpio* was found to be 3.2mg/L for Cadmium and .38mg/L for Hg (table 5 and 6). Mercury and Cadmium both are non essential and poisonous metals. In the present study it is evident that low LC 50 value of Hg shows that it is more toxic than Cadmium in *C. carpio*. The susceptibility of fish to a particular heavy metal is very important factor for LC 50 values. The fish that is highly susceptible to toxicity of one metal may be less or non susceptible to toxicity of another metal at the same concentration. Similarly the metal which is highly toxic to one organism at low concentration may be less or non toxic to other organism.

Gupta and Rajbansh (1981) reported that LC 50 value of Copper with 4.17 and .85 ppm have been calculated in *Mystus bleckeri* at 24h and 96 h. Similar results have been observed in present study. In fig 6, 12 and 18, decrease in LC 50 value has been observed by increasing duration of exposure. In present study the 96h LC50 value has been calculated to be 22.4 mg/L in *H. fossilis* for Copper. The high LC 50 value of Copper in *H. fossilis* showed that the fish is highly susceptible for Copper metal.

5.2 Behaviour Abnormality in Fish Due to Effect of Metals:-

Behavioral changes in animals are indicative of internal disturbance of body function such as inhibition of enzyme function (Cearley 1971) and disruption of the metabolic activity. The present study has been conducted to investigate the behavioral abnormalities in fish *C. carpio* on its exposure to Cadmium and Mercury and Cat fish *H. fossilis* on its exposure to Copper treatment. It is evident from the observations that the behavioural changes in fish *C. carpio* due to both heavy metals have the same pattern of effects. The lowest concentration of both Mercury and Cadmium did not cause any significant change in fish. The second higher concentration in 1.5 mg/L has resulted in the increase of swimming activity and breathing rate. Highest concentration caused lethargic condition and loss of equilibrium in exposed animals (Table 9 and 10). Behavioral changes in Copper treated *H. fossilis* have also been investigated. The low concentration treatment of Copper effect the increase in locomotion and opercular movement. In higher concentration *H. fossilis* showed lethargy and loss of equilibrium (Table 11). Similar behaviour alteration in various fish on exposure to

heavy metals have been noticed by several workers. Holcombe et. al. (1976) observed hyperactivity, erratic swimming and loss of equilibrium in brook trout *Salvalinus fontinalis* in response to Lead treatment. *Notemilgonus crysoleucus* when exposed to 6ppm Copper, became restless, sluggish and finally loss of equilibrium was observed (Lewis and Lewis 1971). *Etroplus maculatus* on being exposed to Copper, Mercury and Selenium showed irregular swimming, frequent surfacing, gulping of air and accelerated ventilation with rapid opercular and mouth movement (Veena et.al 1997). Singh and Reddy (1990) also observed the lethargic response and frequent surfacing alone with gulping of air in .25ppm Copper. Behaviour alteration have attributed to nervous impairment due to blockage of nerve transmission (Nilagu 1979).

Somsundarm et. al. (1984) found out that Zinc altered egg incubation time and caused egg, jaw and spinal deformities. Ellgard and Guillpt (1988) reported that metal effected the energy path way, which resulted in depletion of energy. In case of present study the small change in behaviour were observed in low concentration of Cadmium and Mercury. Low concentration of Cadmium and Mercury showed avoidance

of fish to metal, increase in swimming activity with increase in breathing rate, lethargic condition and loss of equilibrium. In *C. carpio* exposed to high concentration of Cadmium and Mercury probably effect metabolic reaction which result in the depletion of energy. Lethargy and loss of equilibrium may be due to depletion of energy in the body of fish. Glucose is the main source of energy. Varying level of blood glucose are indicative of abnormal carbohydrate metabolism. Singh and Raddy (1900) reported the count of glucose which increase in the blood of Indian cat fish *H. fossilis* (Bloch) after 24 hours exposure to low concentration of Copper (250ug/L). Tewari et. al. (1987) reported hyperglycemia in Lead exposed *Barbus conchoniis*.

5.3 Alteration of Growth Due to Effect of Heavy

Metals :-

All the metals exposure induced the changes in fish growth. Growth fluctuations in animals have been observed due to disturbance of body function and disturbance in the metabolic path ways. The present study was conducted to investigate the growth abnormalities in fish *C. carpio* (Linn.) on exposure to Cadmium and Mercury treatment and Cat fish *H. fossilis* (Bloch) on exposure to Copper treatment. It is evident

from the observations, that the growth changes in fish *C. carpio* and *H. fossilis* due to heavy metals have the same pattern. In comparison to control, metal exposed fish show slow growth rate. In fish *C. carpio* and *H. fossilis*, exposed to high concentration of metals fishes show negative growth showed in Table 15, 16, 17 and fig-49, 50, 51. Same observation has been reported by Palanichanathan et. al. (1996). They revealed that rate of food intake, absorption and metabolism decreased from that control value. Amongst *M. vittatus* exposed to Dimecron and Thiodon the fish exhibited lower growth rate at 42ppm Dimecron and .00006 ppm Thiodon, but negative growth has been recorded at 125ppm Dimecron and .00018ppm Thiodon. Shukla et. al. (1987) observed survivality and impaired growth in Arsenic treated fingerlings of *Channa punctatus*, a fresh water murrel. At a long time exposure to 7.0 mg/L Arsenic during the first seven days there does not occur any change of growth in length but the increase in weight decreased by 6.6 and 11.7% respectively till the 31 days. Measurement of change in growth rate have much to be commented, since an organism growing through a normal rate can be presumed to be healthy and it is an important factor for species of commercial importance. Weight gain due to increased fat content is not universally considered true growth.

Some investigators considered that true growth occurs only when there is an increase of protein. However fat storage is important ecologically and bioenergetically because fat can be used as a source of food during the period of maturation and reproduction. The hormones of the thyroid with those of adrenal gland and growth hormone from pituitary, regulate energy utilization, metabolic rate and growth. Some studies have shown that thyroid activity can be affected by endosulphan, organic and inorganic Mercury and Lead (Kime 1998).

5.4 Histological Changes in the Selected Organs of Fish due to Effect of Metals :-

5.4.1 Histological Changes in the Gill of Fish Due to Effect of Metals :-

The highly branched structural organization of gill and the resultant highly increased surface area, along with the large volume of water passing through the gill surface and highly vascular physiological state of a fish has relatively small biomass, when compared to their surface area (Mayer et. al. 1991) The gill are the primary respiratory organ of fish. The gill epithelium of fish is the major site of gaseous, acid base balance, ionic regulation and excretion of nitrogenous waste.

Histology of gill shows typical structural organization of lamella in untreated *C. carpio* (P-3) and *H. fossilis* (P-10). There are several alterations which have been noticed in *C. carpio* exposed to Cadmium and Mercury in the present study, such as hyperplasia and hypertrophy of chloride cell and mucus cells, edema of epithelial cells, clumping of gill filament, aneurism and shortening of secondary lamella by fusion (P-4, 5, 6, 7, 8 and 9). *H. fossilis* exposed to Copper sulphate showed hyperplasia of primary lamellar epithelium, hyperplasia of secondary lamellar epithelium, lamellar telangiectasis, hyperplasia of chloride cells and epithelium lifting (P-11, 12, 13). Several investigations have reported histological changes in the gills of different fish exposed to Heavy metal. Kalele and Dhanda (2005) has reported the tissue necrosis, ruminant rupture and fusion of secondary lamellae in the gill in Copper sulphate exposed *Labeo rohita*. Ahmad and Datta Munshi (1987) reported the histological changes in gill, such as pronounced epithelial lifting and enlarged chloride cells in Copper exposed *Labeo rohita* and *Catla catla*. Alteration of epithelial surface of gill has been reported in *Dania dangla* due to Copper (Ojha and Singh 1986). Crespo and Sala (1986) observed ultra structural changes in gill filament as proliferation and alteration of chloride

cell in *Scyliorhinus canicula* treated with Copper for two weeks. Griffit et. al. (2007) investigated the effect of nanocopper on the gill of Zebrafish exposed to 100ug/L of nanocopper. They revealed that gill was the primary target organ for nanocopper which alter the histology and biochemistry of gill.

5.4.2 Histological Changes in the Liver of Fish Due to

Effect of Metals :-

Liver histology shows typical structural organization of hepatocyte cells in untreated *C. carpio* (P-14) and *H. fossilis* (P-20). The treated *C. carpio* liver displayed the highest prevalence of histological changes, with necrosis, representing the dominant structural alteration (P-16, 17). Hepatic necrosis and inflammation is indicative of infection and toxic injury by contaminants, which has been observed in green back flounders exposed to contaminated marine sediment (Mondon et. al. (2001). In addition, hepatocyte of *C. carpio* exposed to heavy metal showed hypertrophy, swelling and nuclear pyknosis. In more severe cases, there was slight blood congestion in sinusoid (P-19). Similar alteration were observed in the hepatocytes of Nile Tilapia exposed to glyphosate herbicide (Jiraungkoorskul et. al. 2003). Copper treated *H. fossilis* liver showed irregular

clumping of cells (P-21). Prolonged exposure showed lysis of liver cell (Rawat et. al. 2002). Hepatic cells damage in the liver of the *H.fossilis* has been reported, when fish has been exposed to endosulphan pesticide. Histological change such as hepatic lesion and necrosis in liver has been observed in *Gambusia affinis* exposed to Dimecron pesticide for 30 day (Saktivel, Veena and Gaikwad 2002).

5.4.3 Histological Changes in the Kidney of Fish Due to Effect of Metals :-

Kidneys histology shows typical structural organization of proximal tubule, glomeruli and nucleus in the untreated *C.carpio* (P-23) and *H. fossilis* (P-29). The *C.carpio* exposed to Cadmium and Mercury and *H.fossilis* treated with Copper show similar histopathological alterations. Kidney lesion consisting of dilation of Bowman's space and accumulation of hyaline droplet in tubular epithelial cells have been observed in *C.carpio* (P-25) and *H.fossilis* (P-30) due to metal treatment. Similar observations have been reported by Piyanut et. al. (2006) in Nile Tilapia. Swelling and hypertrophy of proximal tubular cells and nuclear pyknosis has been observed in *C.carpio* (P-26, 27, 28) and *H.fossilis* (P-31).

5.5 Bioaccumulation of Metals in Selected Organs of Fish:-

The present observations revealed that tissue wise accumulation of each heavy metal varied in *C. carpio* for Cadmium in the order Gill > Liver > Kidney (Fig-52) and for Mercury Liver > Gill > Kidney (Fig-53). Copper accumulation in the tissue of *H. fossilis* was found to be in the order Liver > Gill > Kidney (Fig- 54). The Mercury is found to be more toxic than Cadmium so Mercury is less accumulated in tissue in comparison to Cadmium. This difference in accumulation may be attributed to the physiological state of tissue, presence of ligand having an affinity to metal and/or tissue in the detoxification and the proximity of the tissue to the toxicant medium. The results presented in Table 12, 13, and 14 showed that in general, heavy metals are more accumulated in liver than in gill. Gill contains lower level of heavy metal than liver except in the case of Cadmium. The concentrations of heavy metal are lowest in kidney. The different degree of the metal accumulation in various tissues depend on their biochemical characteristic (Farkas et.al.2000). Target organs such as Liver and Gill, are metabolically active tissue and accumulate heavy metal in higher

level as was observed in experiments (Allen 1995; Kalay and Erden 1995; Tulasi et. al. 1992; Allen 1994) and field study (Langston 1990, Spehar et. al. 1982).

The available literature justifies that accumulation of metals in the tissue of fish is dependent upon exposure concentration and duration as well as factor such as salinity, temperature, hardness and metabolism of animal (Pagenkopt 1983; Allen 1995). Agarwal et. al. (2007) reported Mercury and Lead content in fish species from the river Gomti, Lucknow, India. They showed that the accumulation pattern of Mercury in fish species examined was, in order *M. armatus* (Bam) > *C. batrachus* (mangur) > *M. cavasius* (Tengar) > *N. notopterus* (Patra) > *R. rita* (belgegra) \cong *H. fossilis* (Singhi) > *C. punctatus* (Girai) > *L. rohita* (rohu). They also showed that the concentration of Lead in different species of fish examined was in following order of accumulation. *C. batrachus* (mangur) > *M. armatus* (Bam) > *C. punctatus* (Girai) > *L. rohita* (rohu) > *H. fossilis* (Singhi) > *M. cavasius* (Tengar) > *N. notopterus* (Patra) > *R. rita* (belgegra).

Liver can accumulate the highest level of metal concentration, followed by gill and kidney (Chen and Chen 1999; Farkas et. al. 2000; Lundebye et. al. 1999). Our similar results

have been observed except in the case of Cadmium. Liver play an important role in storage, redistribution of contaminant and also act as an active site of the pathological effect (Evans et. al.1993) According to Jaffar and Shahid (1989) liver is an organ where the specific metabolic processes and enzyme catalyzed reaction related to these metals take place. The liver contains highest metal concentration because it is an organ for storage and detoxification (Avenant, Oldewage and Mark 2002). The liver tissue are more often recommended as an indicator of water pollution than any other organ of fishes. (Al-yousuf et. al. (2000). Fish gill is the tissue that is more often found to have high heavy metal concentration (Laundebye 1999; Farkas et. al. 2000; Romeo et. al. 1999). Fish may accumulate heavy metals by absorption through gills (Wong et.al 2001) .The gill act as the primary site of metal accumulation, by absorption in the present study. It is external in position and its proximity to ambient toxicants is close. In addition to its highly branched structure and organization of the gill and the resultant highly increased surface area, along with large volume of water passing through the gill surface makes it more vulnerable. The highly vascular physiological state and the relatively small biomass when compared to their surface area (Mayer et.al. 1991) makes the

gill a prime site for metal accumulation. According to Dianne et. al. (1999) after absorption, Cadmium is transported in the blood and bounded to albumin. It is taken up by Liver, where it induces the synthesis of Metallothionein (MT), to which it binds. Cadmium MT is released into circulation, filtered by the kidney and reabsorbed by endocytosis in to the cells of proximal tubules. According to Klavercamp et. al. (1984) the Gill, Liver and Kidney, are the main site of Metallothionein production and metal reaction. This may be the main reason for the enhanced presence of metal in the gill, Liver and Kidney. In addition, all these tissues are rich in the Cadmium bind-SH group (Rama and Philip 1997) and therefore it is not surprising that metal ions are completed in these organs.

Liver and kidney are involved in detoxification process and the removal of toxic substances circulating in blood stream (Kent 1998). According to Klavercamp et.al (1994), metal might also be transported into these organs from other tissue including gill, for the purpose of subsequent elimination. Such transportation leads to higher rates of accumulation in these organs (Liver and Kidney). Unbounded metal such as Cadmium and Mercury, can be reabsorbed by active transport mechanism

in the cells of proximal convoluted tubules. Once they are in the cell, they bind with metallothionein, resulting in their accumulation (Dornian and Gatton (1992). All these observations justify the possibility of transport in the trace amount of metal from various tissue to kidney.

CHAPTER -6

CONCLUSION

6.1. Conclusion:-

In the present work the toxic stress caused by acute and chronic exposure of Cadmium, Mercury and Copper has been observed. Selected parameters such as alteration in growth, behavioural changes, bioaccumulation and histopathological abnormalities in the Gill, Liver and Kidney of *Cyprinus carpio* and *Heteropneustes fossilis* have been studied.

Acute toxicity test demonstrated the effects in short period (4d) of exposure and chronic toxicity test concluded the effect for relatively longer period of time. By acute toxicity test it is concluded that increase in the mortality of fish is observed by increasing the concentration and increasing the time period. Higher LC50 value show that metal is less toxic because great concentration is required for 50% mortality of the organism. In the present study it has been observed that Mercury is highly toxic than Cadmium for *Cyprinus carpio* and Copper is least toxic for *Heteropneustes fossilis* because the LC 50 value of Copper is higher than Mercury and Cadmium. By increasing the time period the value of LC 50 was found to be always decreasing. The LC50 value is highest at 24h and lowest at 96h.

Heavy metals directly influence the behaviour of the fish by impairing neurological functions. Metal influence neurotransmitter production and the alteration of metabolic processes. Lethargy and loss of equilibrium which has been observed, may due to depletion of energy in the body of animal. The impairment of carbohydrate metabolism in heavy metal exposed fish has been reported by several workers. Donaldson and Dye (1975) reported the release of corticosteroid hormone in *Sockeyes salmon* when treated with Copper. Behavioural changes in fish *C.carpio* and *H.fossilis* for three metals have the same pattern of effect. The lowest concentration of metal Cadmium, Mercury and Copper can not cause any significant change in behaviour. In second higher concentration of metal treatment increased swimming activity has been observed with higher breathing rate and more higher concentration of metals caused lethargy and loss of equilibrium.

Growth fluctuation in animal is due to disturbances of body functions and disturbance of body's metabolic path way. Present study was conducted to investigate the growth abnormality in fish *C.carpio* (Linn.) exposed to Cadmium and Mercury and *H.fossilis* (Bloch) on exposure to Copper treatment. It was noticed that fish growth rate reduced by increasing the

concentration of metal. It is evident from the observations that the change in growth of fish *C. carpio* and *H. fossilis* due to all heavy metals have the same pattern of effect. In comparison to control fish metal exposed fish show slow growth rate. In higher concentration of metals *C. carpio* and *H. fossilis* fishes show negative growth.

Changes in histological structure in specific vital organs due to exposure of sub lethal concentration of metal in various fishes have been reported by several workers. Gills are the primary respiratory organs of fish. The gill epithelium of the fish is major site of gaseous exchange, acid base balance, ionic regulation and excretion of nitrogenous waste. Gills are the primary target organs for water born toxicant such as heavy metals. Liver plays major role in metabolism of excretion, digestion and storage of various substances including metal substance of fish. An investigation on the effect of heavy metals Cadmium, Mercury and Copper on gill, liver and kidney of *C. carpio* and *H. fossilis* was carried out in the laboratory. The result show that degree of distortion of gill, liver and kidney was proportionate to exposure period and concentration of metal and that is it was dose and time dependent.

Heavy metals enter and accumulate in the body tissue faster than body detoxification path way and gradual build up accumulation of these toxic metals occurs. It has been observed that accumulation of metals varies in various tissue of fish.

6.2 Further scope of research:-

The present investigations suggest that :

1. Combination of metal may be taken to study the toxic effect of mixture of metals on fish.
2. Other metals and other economically important species of fish may be taken to study the toxicity of metals on fish.
3. Further study may be undertaken to assess the toxic effect of metal on fish at molecular level (alteration of gene).
4. Eggs and larval stage of fish may be used as test organism to ascertain the toxic effect of metal on early life stages of fish.
5. Effect of metal on physiology of blood serum may be studied because blood is a good physiological indicator.
6. Further work may be under taken to study the effect of metal on endocrinal glands and harmones of fish.

7. Heavy metals tend to reach the aquatic medium from their sources and further take their way to human being through the aquatic life. Some experiments have been arranged to check the passage of the heavy metals to reach the human body and the removal of metal in aquatic medium, which may be taken up for further research.
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TABLES

Table-: 1 Permissible limits for various metals in drinking water. (Industrial Toxicological Research Center (2000).

Metal	Symbol	Prescribed Limit(mg/L)
Arsenic	As	.05
Cadmium	Cd	.005
Chromium	Cr	.05
Copper	Cu	.05
Iron	Fe	.03
Lead	Pb	.05
Manganese	Mn	.1
Mercury	Hg	.001
Nickel	Ni	-
Zinc	Zn	5.0

Table -:2 Physico chemical properties of water.

Sl.No	Parameters	Range	Mean values
1.	Temperature $^{\circ}\text{C}$	24-26	25
2.	pH	6.8-7.4	7.1
3.	Dissolved Oxygen (DO) mg/L	6.9-7.8	7.3
4.	Hardness (mg/L as CaCO_3)	186-238	212

Table:-3 Percentage of mortality in *C. carpio* (Linn.) due to different concentration of Cadmium chloride in different exposure duration.

Conc. of CdCl ₂ (mg/L)	No. of test organism	Percent mortality				
		24h	48h	72h	96h	120h
Control	10	0	0	0	0	0
3.0	10	0 (0)	20 (4.1)	30 (4.4)	40 (4.7)	50 (5.0)
3.5	10	20 (4.1)	30 (4.4)	40 (4.7)	60 (5.2)	60 (5.2)
4.0	10	30 (4.4)	40 (4.7)	60 (5.2)	70 (5.5)	80 (5.8)
4.5	10	50 (5.0)	60 (5.2)	70 (5.5)	80 (5.8)	90 (6.2)
5.0	10	60 (5.2)	70 (5.5)	80 (5.8)	90 (6.2)	90 (6.2)

Probit mortality in bracket.

Table:-4 Percentage of mortality in *C. carpio* (Linn.) due to different concentration of Mercuric chloride in different exposure duration.

Conc. of HgCl ₂ mg/L	No. of test organism	Percent mortality				
		24h	48h	72h	96h	120h
Control	10	0	0	0	0	0
.30	10	10 (3.7)	20 (4.1)	30 (4.4)	30 (4.4)	50 (5.0)
.35	10	20 (4.1)	30 (4.4)	40 (4.7)	40 (4.7)	60 (5.2)
.40	10	30 (4.4)	40 (4.7)	50 (5.0)	60 (5.2)	70 (5.5)
.45	10	40 (4.7)	60 (5.2)	60 (5.2)	70 (5.5)	90 (6.2)
.50	10	60 (5.2)	70 (5.5)	70 (5.5)	80 (5.8)	100 (8.0)

Probit mortality in bracket.

Table:-5 LC 50 values of Cadmium chloride on *C. carpio* in different exposure duration (estimated by probit analysis).

Exposure duration	Median lethal concentration (mg/L)
24h	4.5
48h	4.2
72h	3.9
96h	3.2

Table:-6 LC 50 values of Mercuric chloride on *C. carpio* in different exposure duration (estimated by probit analys)

Exposure duration	Median lethal concentration (mg/L)
24h	.46
48h	.42
72h	.40
96h	.38

Table:-7 Percentage of mortality in *H. fossilis* due to different concentration of Copper sulphate in different exposure duration.

Conc. of CuSO ₄ (mg/L)	No. of test Organism	Percent mortality				
		24h	48h	72h	96h	120h
Control	10	0	0	0	0	0
10	10	0 (0)	10 (3.7)	20 (4.1)	30 (4.4)	40 (4.7)
20	10	10 (3.7)	20 (4.1)	30 (4.4)	40 (4.7)	50 (5.0)
30	10	30 (4.4)	50 (5.0)	50 (5.0)	60 (5.2)	70 (5.5)
40	10	40 (4.7)	60 (5.2)	60 (5.2)	70 (5.5)	80 (6.2)
50	10	60 (5.2)	70 (5.5)	70 (5.5)	80 (5.8)	100 (8.0)

Probit mortality in bracket.

Table:-8 LC 50 values of Copper sulphate on *H. fossilis* in different exposure duration (estimated by probit analysis).

Exposure duration	Median lethal concentration (mg/L)
24h	45.0
48h	32.0
72h	28.0
96h	22.4

Table:- 9 Behavioural changes determined in *C. carpio* (Linn.) on exposure to Cadmium chloride treatment for one week (+) indicates an increase.

Parameters	Concentration of Cadmium chloride (mg/L)		
	.32	1.5	3.0
Locomotion	+	+	
Opercular movement	+	+	
Lethargy		+	+
Loss of equilibrium		+	+

Table:- 10 Behavioural changes determined in *C.carpio* (Linn.) on exposure to Mercuric chloride treatment for one week (+) indicates an increase.

Parameters	Concentration of Mercuric chloride (mg/L)		
	.038	.10	.15
Locomotion	+	+	
Opercular movement	+	+	
Lethargy		+	+
Loss of equilibrium			+

Table:- 11 Behavioural changes determined in *H. fossilis* on exposure to Copper sulphate treatment for one week (+) indicates an increase.

Parameters	Concentration of Mercuric chloride (mg/L)		
	2.24	5	10
Locomotion	+	+	
Opercular movement	+	+	
Lethargy		+	+
Loss of equilibrium			+

Table-: 12 Bioaccumulation of Cadmium ($\mu\text{g/g}$) in liver, gill and Kidney of *C .carpio* at various exposure duration of .32mg/L Cadmium chloride.

Bioaccumulation of CdCl_2 in various tissues	Control	Exposure duration in hours		
		240h	480h	720h
Liver	0.00	.038	.040	.038
Gill	0.00	.049	.056	.051
Kidney	0.00	.030	.038	.035

Table-: 13 Bioaccumulation of Mercury ($\mu\text{g/g}$) in liver, gill and Kidney of *C .carpio* at various exposure duration of .038 mg/L Mercuric chloride.

Bioaccumulation of HgCl_2 in various tissues	Control	Exposure duration in hours		
		240h	480h	720h
Liver	0.00	.0051	.0068	.0063
Gill	0.00	.0042	.0048	.0087
Kidney	0.00	.0035	.0037	.0037

Table-: 14 Bioaccumulation of Copper ($\mu\text{g/g}$) in liver, gill and kidney of *H. fossilis* at various exposure duration of 2.24 mg/L Copper sulphate.

Bioaccumulation of CuSO_4 in various tissues	Control	Exposure duration in hours		
		240h	480h	720h
Liver	0.00	30.29	32.38	32.01
Gill	0.00	2.85	2.98	3.15
Kidney	0.00	1.95	2.10	2.24

Table-:15 Changes in growth (weight) of *C. carpio* exposed to different concentrations of Cadmium chloride for 480h.

Concentration of CdCl ₂ (mg/L)	Initial weight (g/fish)	Final weight (g/fish)	% of weight gain	% of weight loss
Control	5.50	6.01	9.27	-
.5	6.01	6.43	6.98	-
1.0	5.15	5.49	6.60	-
1.5	6.12	6.35	3.75	-
2.0	5.89	5.81	-	1.35
2.5	5.69	5.60	-	1.58

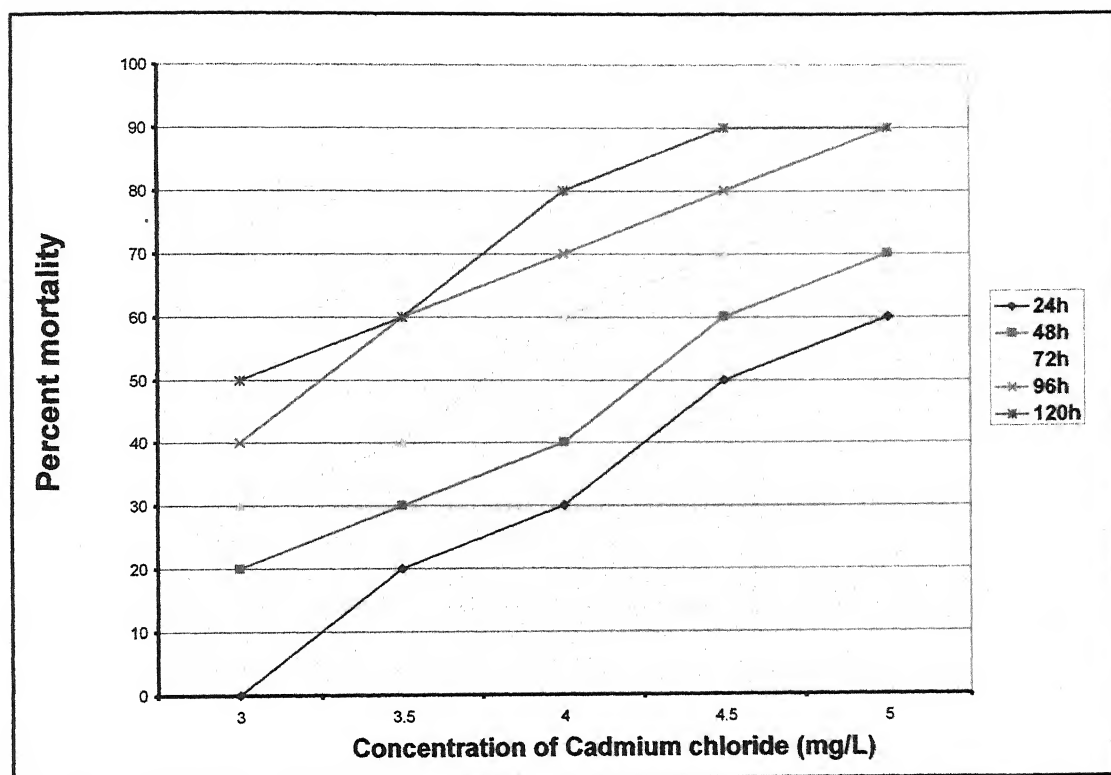
Table-:16 Changes in growth (weight) of *C .carpio* exposed to different concentrations of Mercuric chloride for 480h.

Concentration of HgCl_2 (mg/L)	Initial weight (g/fish)	Final weight (g/fish)	% of weight gain	% of weight loss
Control	5.05	5.58	10.49	-
.10	6.01	6.40	6.48	-
.15	5.89	6.20	5.60	-
.20	5.61	5.88	4.81	-
.25	5.92	5.82	-	1.71
.30	6.08	5.90	-	2.96

Table-:17 Changes in growth (weight) of *H. fossilis* exposed to different concentrations of Copper sulphate for 480h.

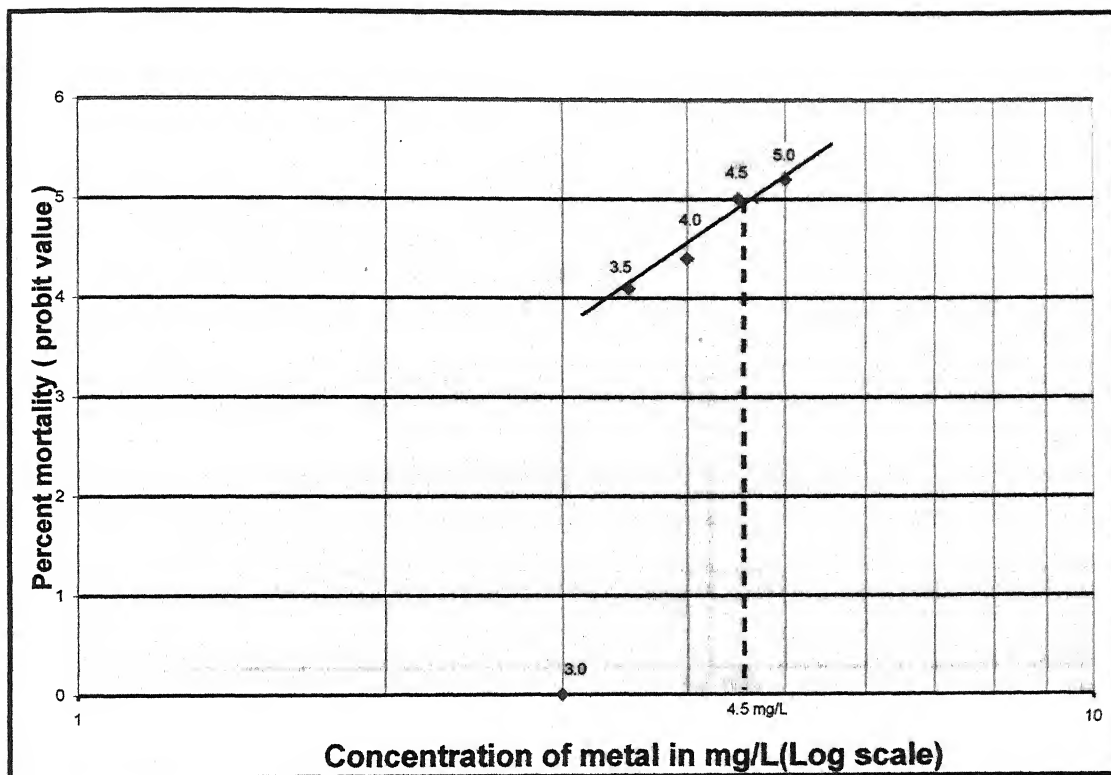
Concentration of CuSO ₄ (mg/L)	Initial weight (g/fish)	Final weight (g/fish)	% of weight gain	% of weight loss
Control	6.50	7.16	10.15	-
1	7.01	7.40	5.56	-
5	6.15	6.45	4.84	-
10	7.12	7.30	2.52	-
15	6.89	6.78	-	1.62
20	6.69	6.52	-	2.54

FIGURES



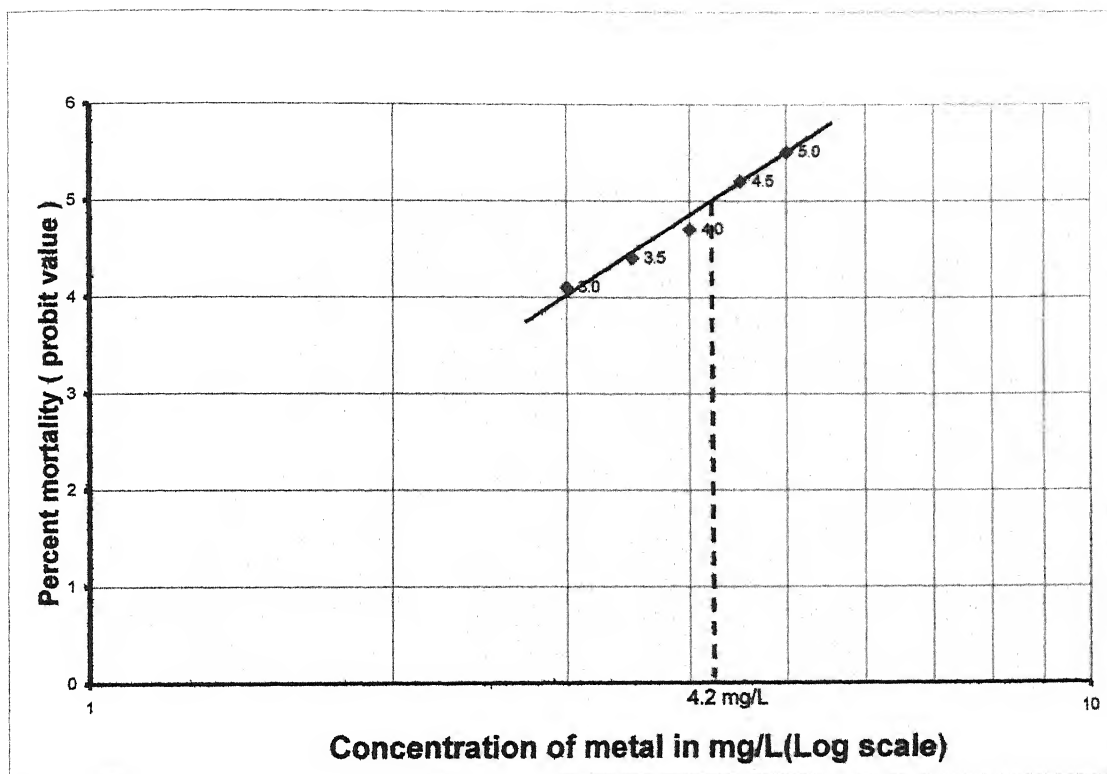
Percent mortality of *C. carpio* (Linn.) in different exposure duration to different concentrations of Cadmium chloride.

Fig-1



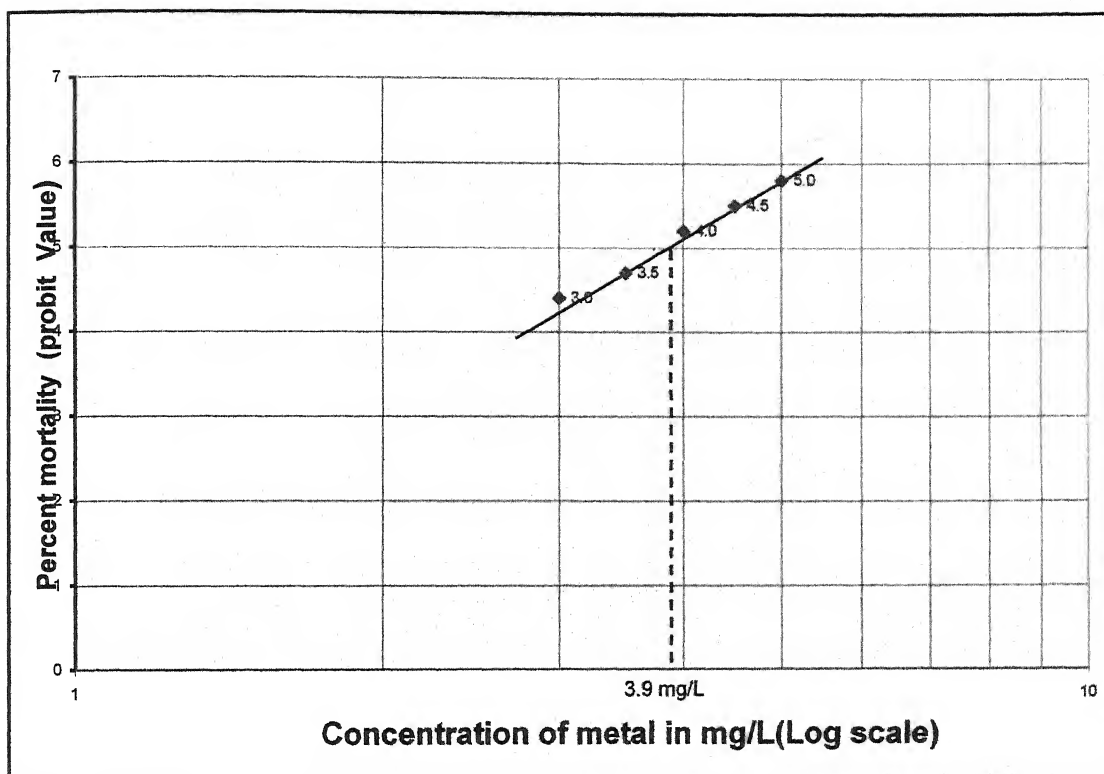
Determination of 24 hrs. median lethal concentration (24h LC 50) of Cadmium chloride to *C. carpio* (Linn.) by probit analysis.

Fig-2



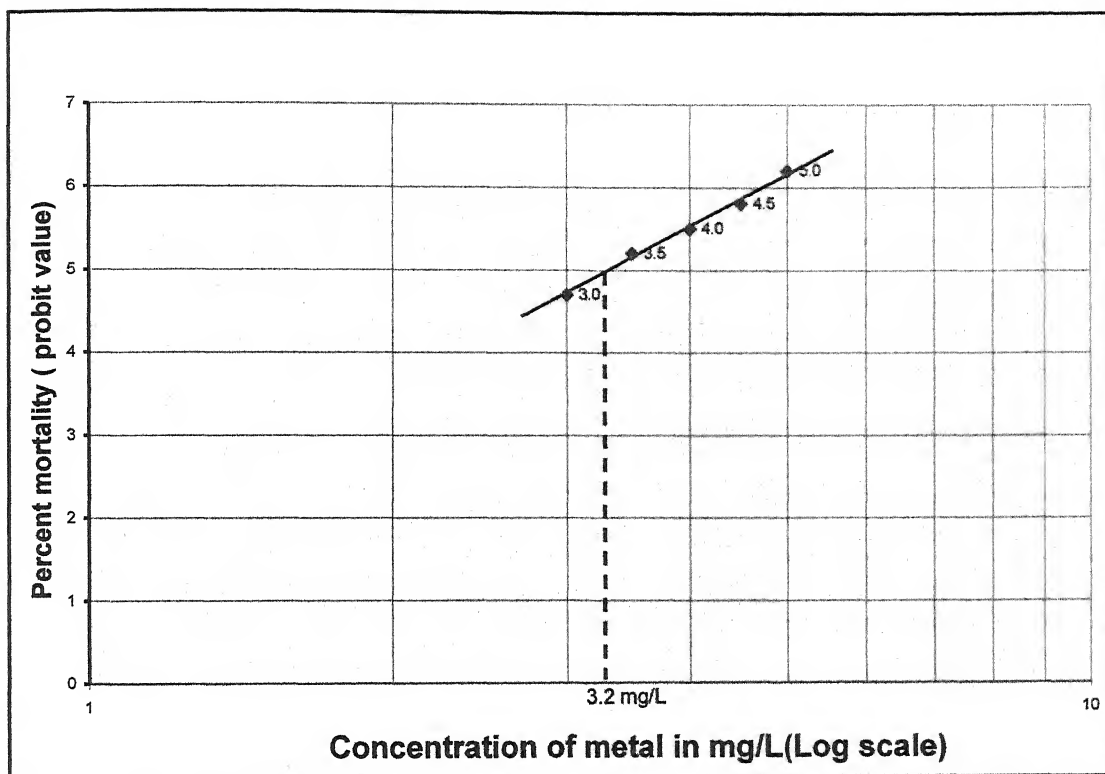
Determination of 48 hrs. median lethal concentration (48h LC 50)
of Cadmium chloride to *C.carpio* (Linn.)by probit analysis.

Fig-3



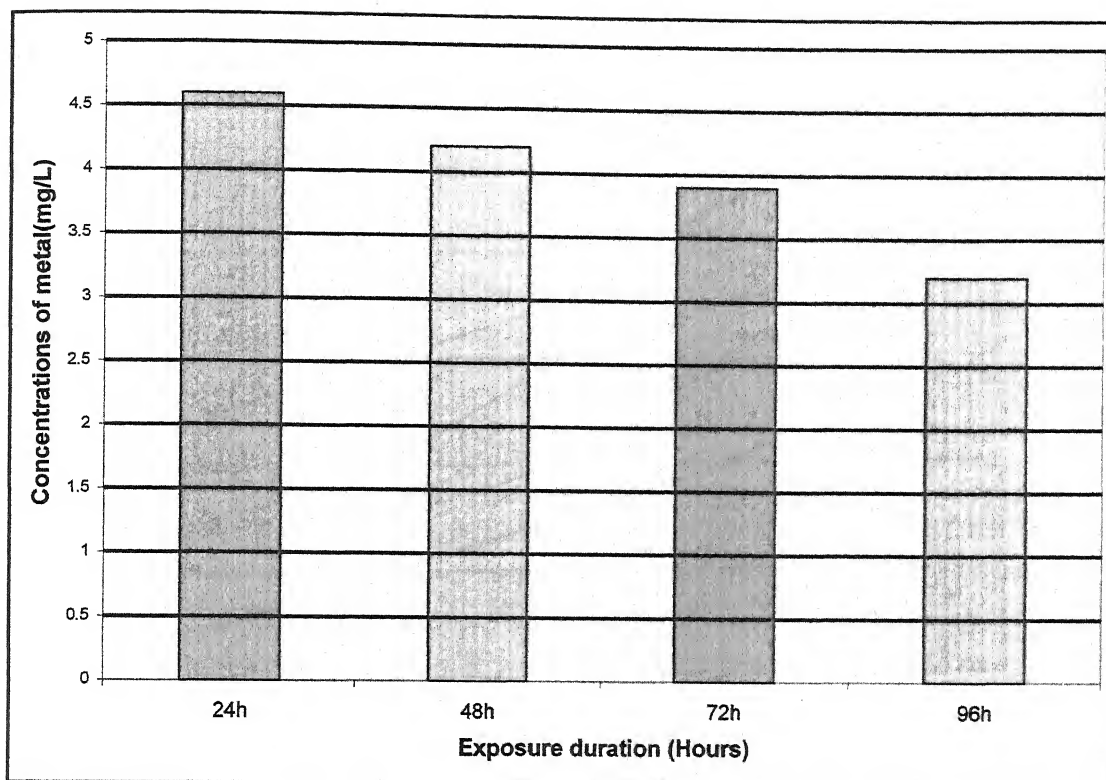
Determination of 72 hrs. median lethal concentration (72h LC 50)
of Cadmium chloride to *C. carpio* (Linn.) by probit analysis.

Fig-4



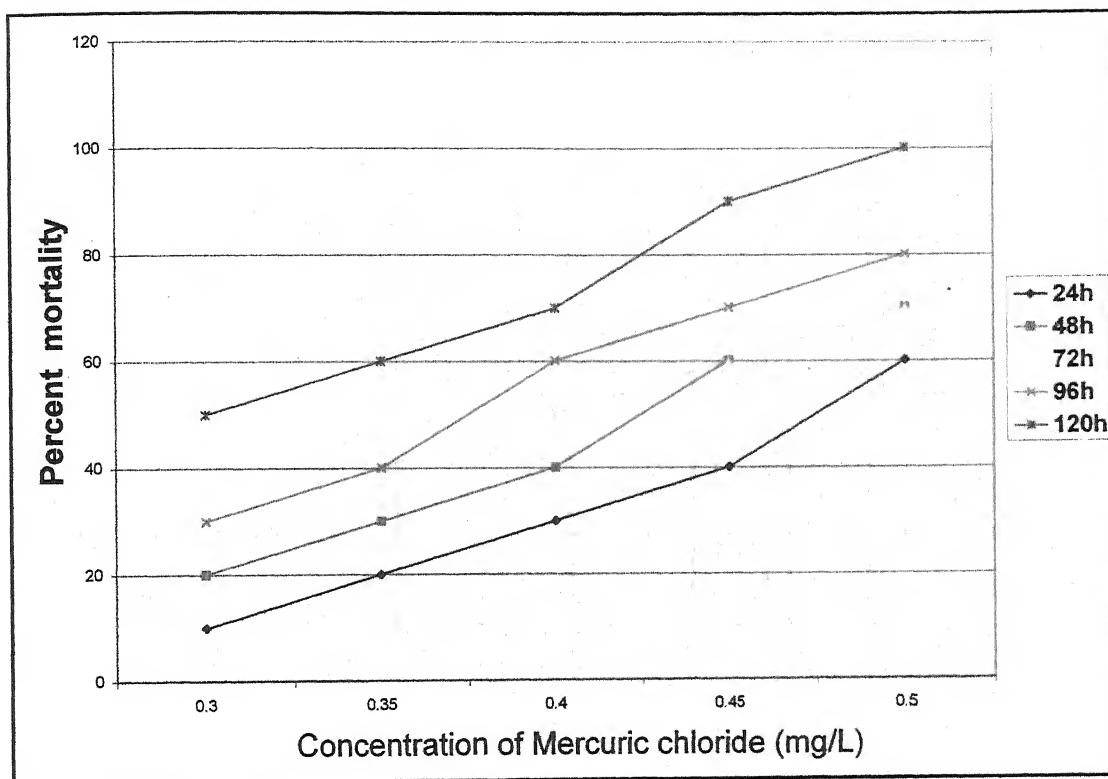
Determination of 96 hrs. median lethal concentration (96h LC 50)
of Cadmium chloride to *C. carpio* (Linn.) by probit analysis.

Fig-5



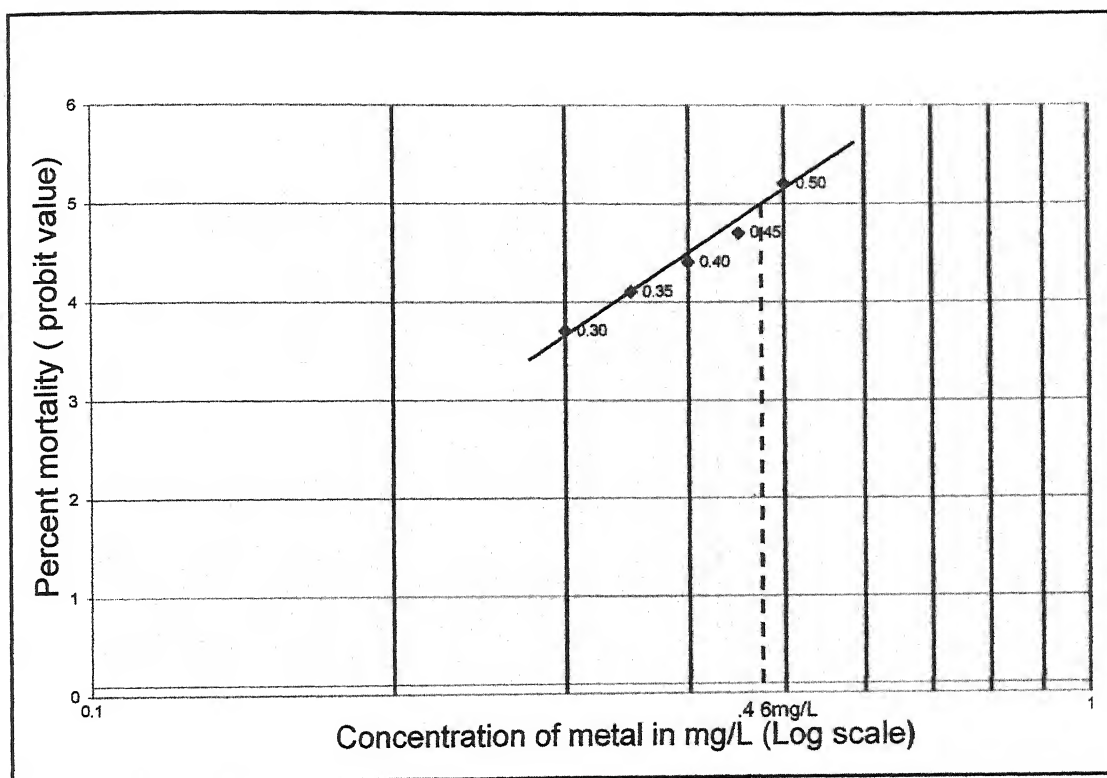
**LC 50 values of Cadmium chloride in different exposure period
in *C. carpio* (Linn.)**

Fig-6



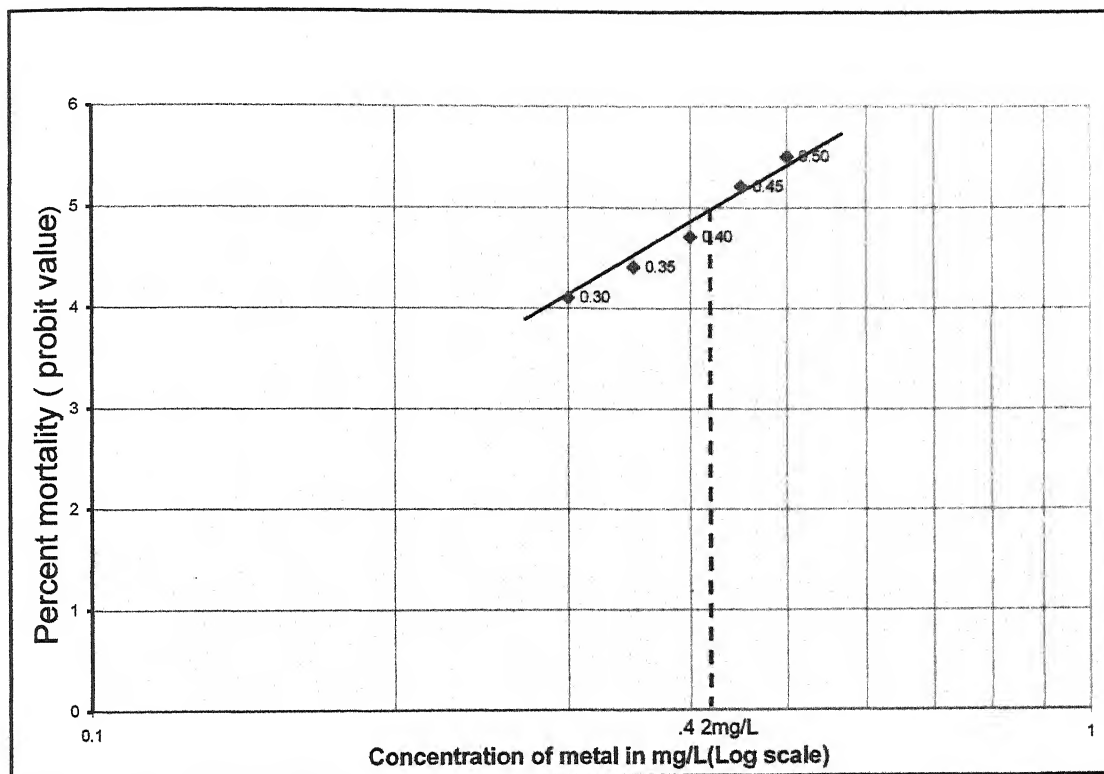
Percent mortality of *C. carpio* (Linn.) in different exposure duration to different concentrations of Mercuric chloride.

Fig-7



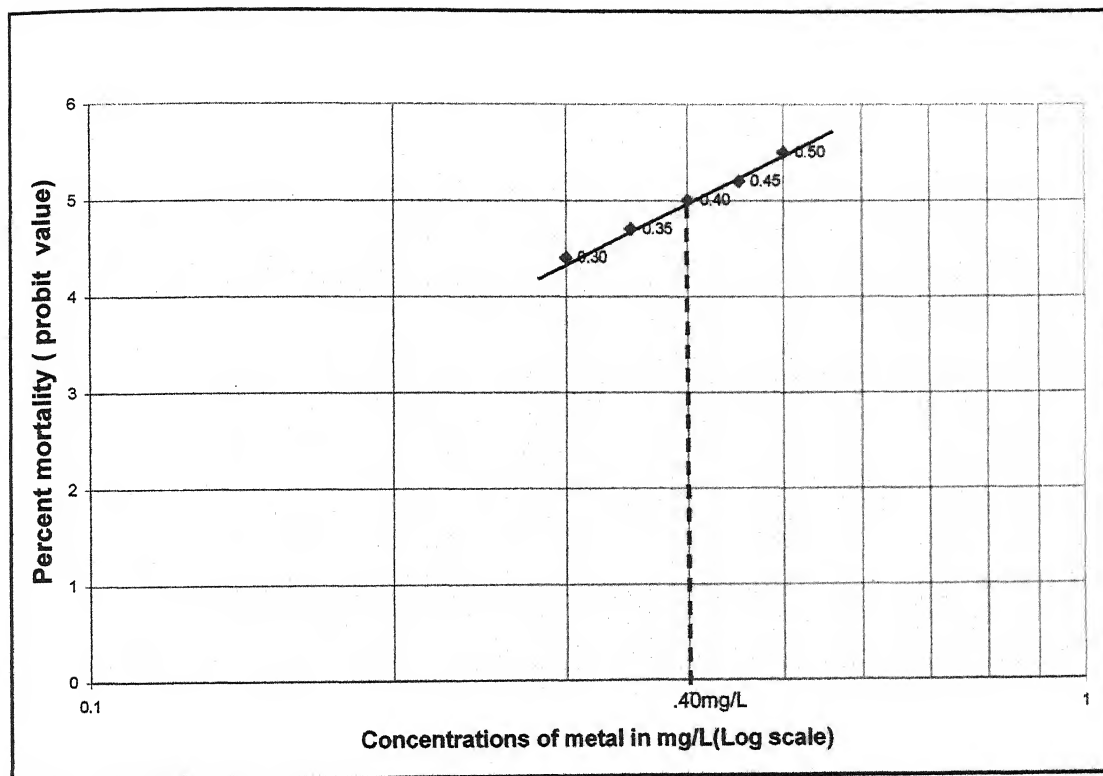
Determination of 24 hrs. median lethal concentration (24h LC 50)
of Mercuric chloride to *C. carpio* (Linn.) by probit analysis.

Fig-8



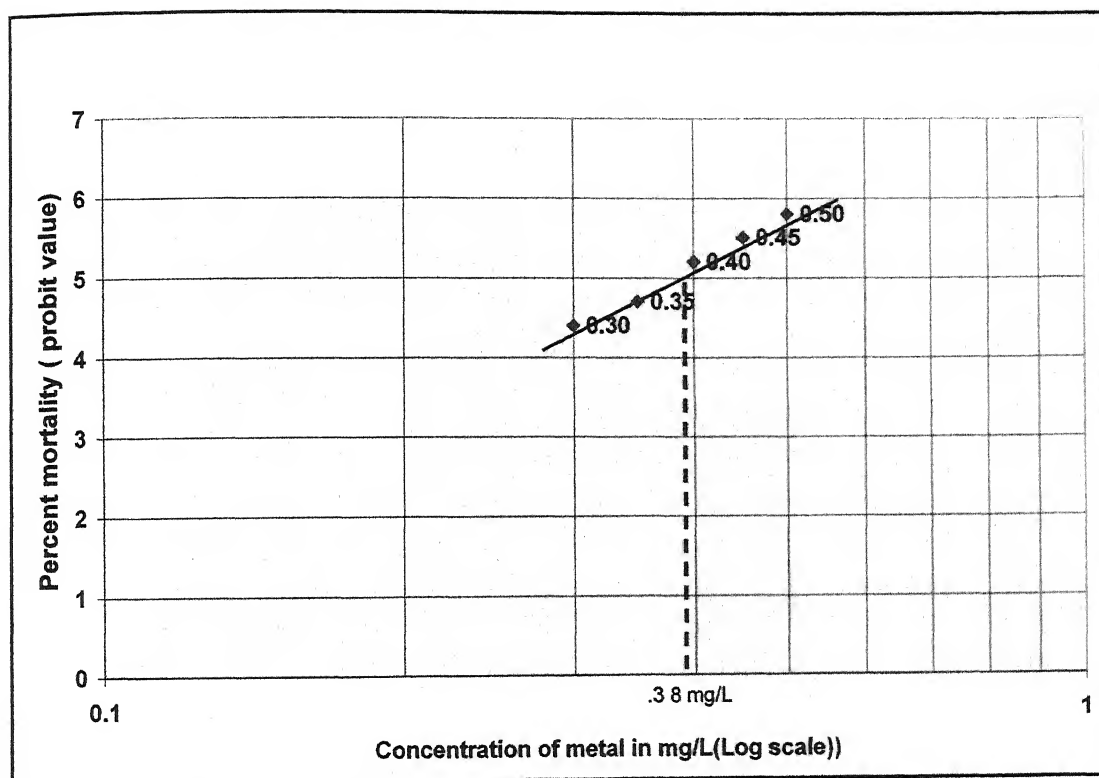
Determination of 48 hrs. median lethal concentration (48h LC 50)
of Mercuric chloride to *C. carpio* (Linn.) by probit analysis.

Fig-9



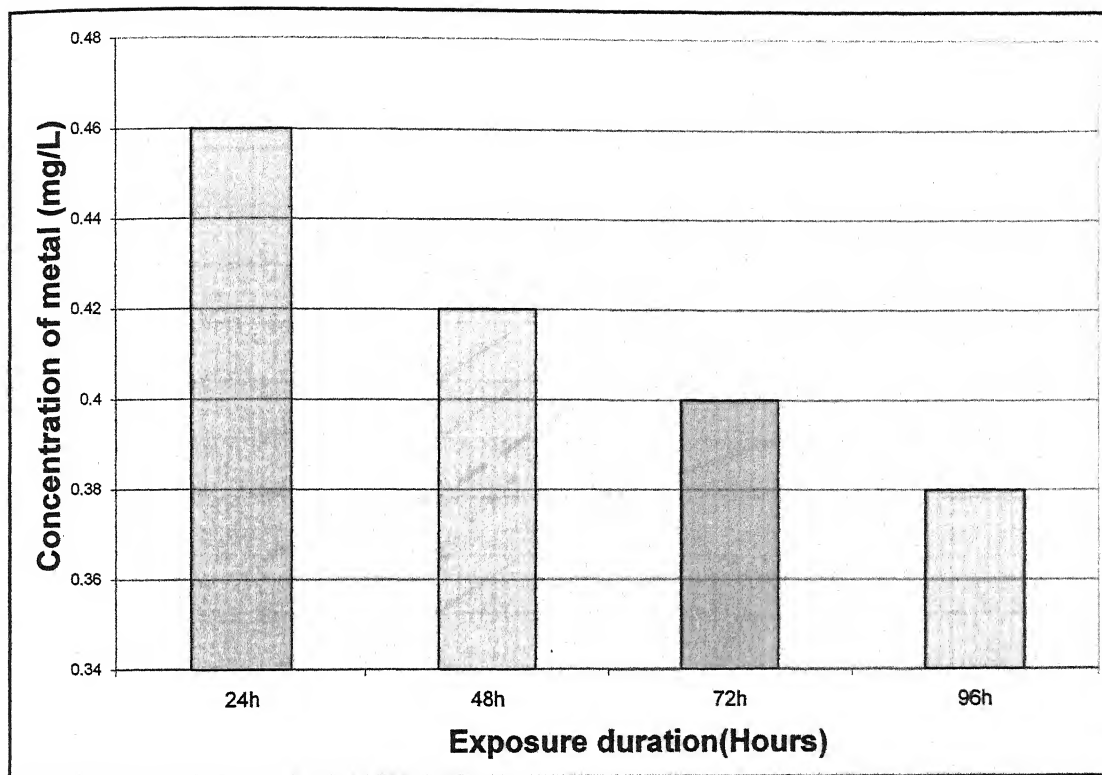
Determination of 72 hrs. median lethal concentration (72h LC 50)
of Mercuric chloride to *C. carpio* (Linn.) by probit analysis.

Fig-10



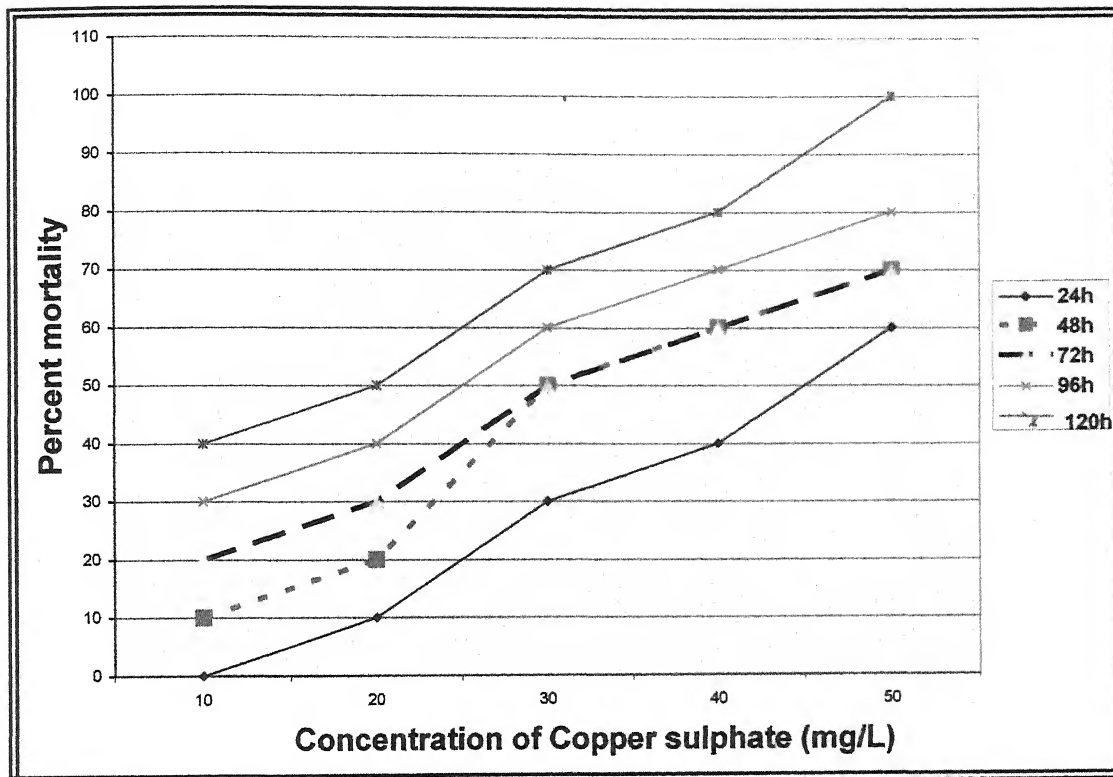
Determination of 96 hrs. median lethal concentration (96h LC 50)
of Mercuric chloride to *C. carpio* (Linn.) by probit analysis.

Fig-11



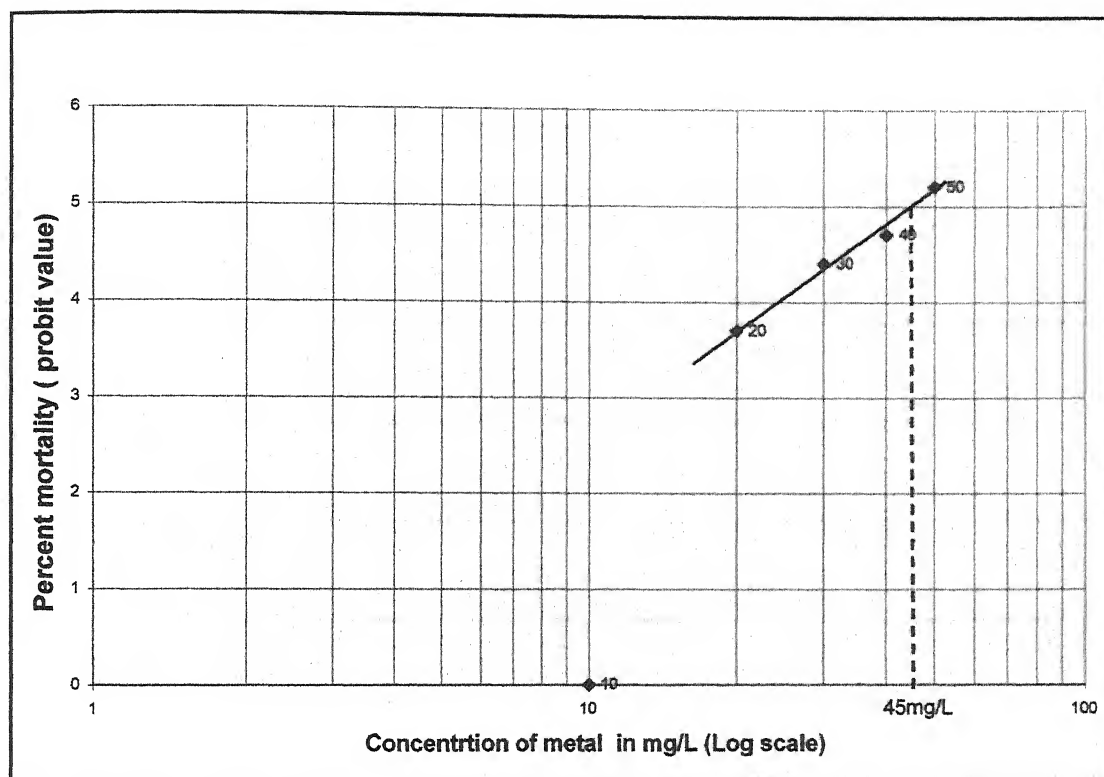
LC 50 values of Mercuric chloride in different exposure period in *C. carpio*(Linn.).

Fig-12



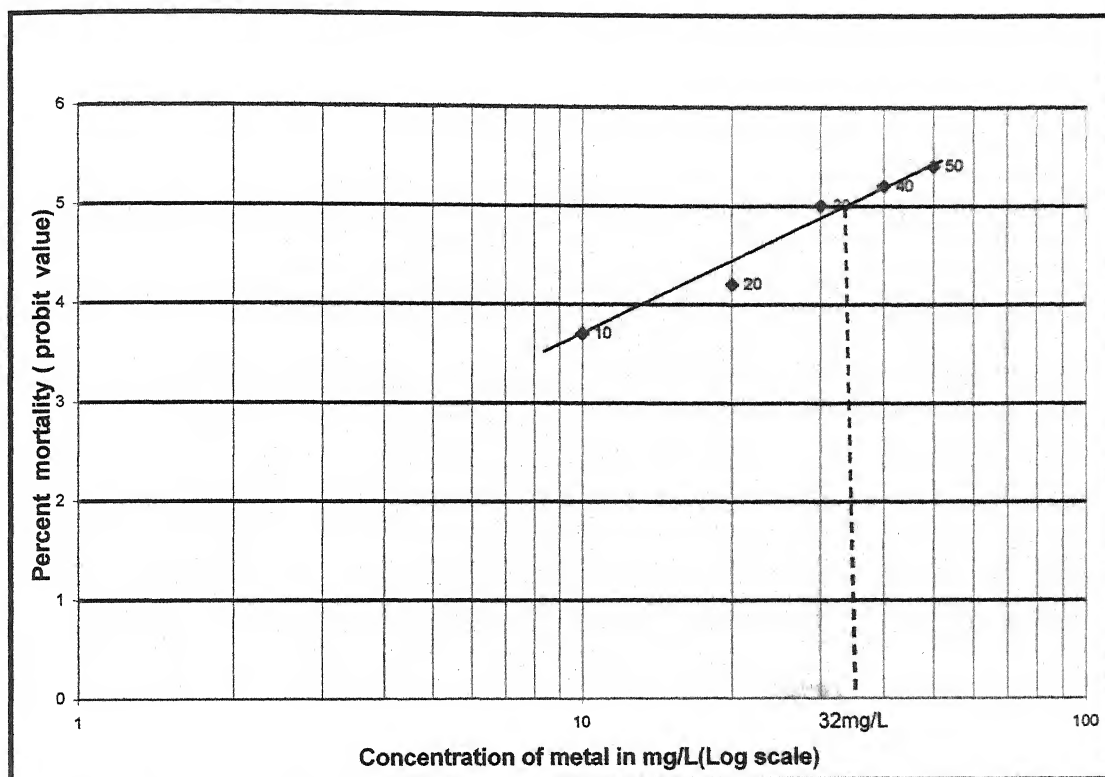
Percent mortality of *H. fossilis* (Bloch) in different exposure duration to different concentrations of Copper sulphate.

Fig-13



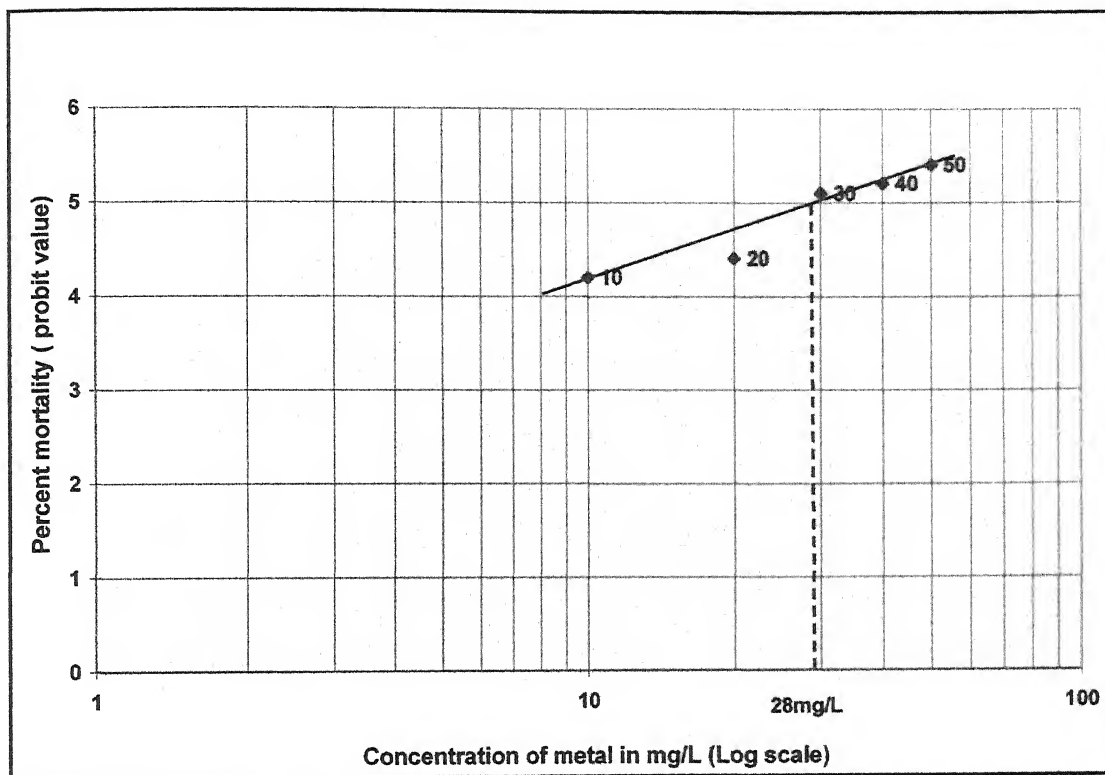
Determination of 24 hrs. median lethal concentration (24h LC 50)
of Copper sulphate to *H.fossilis* (Bloch) by probit analysis.

Fig-14



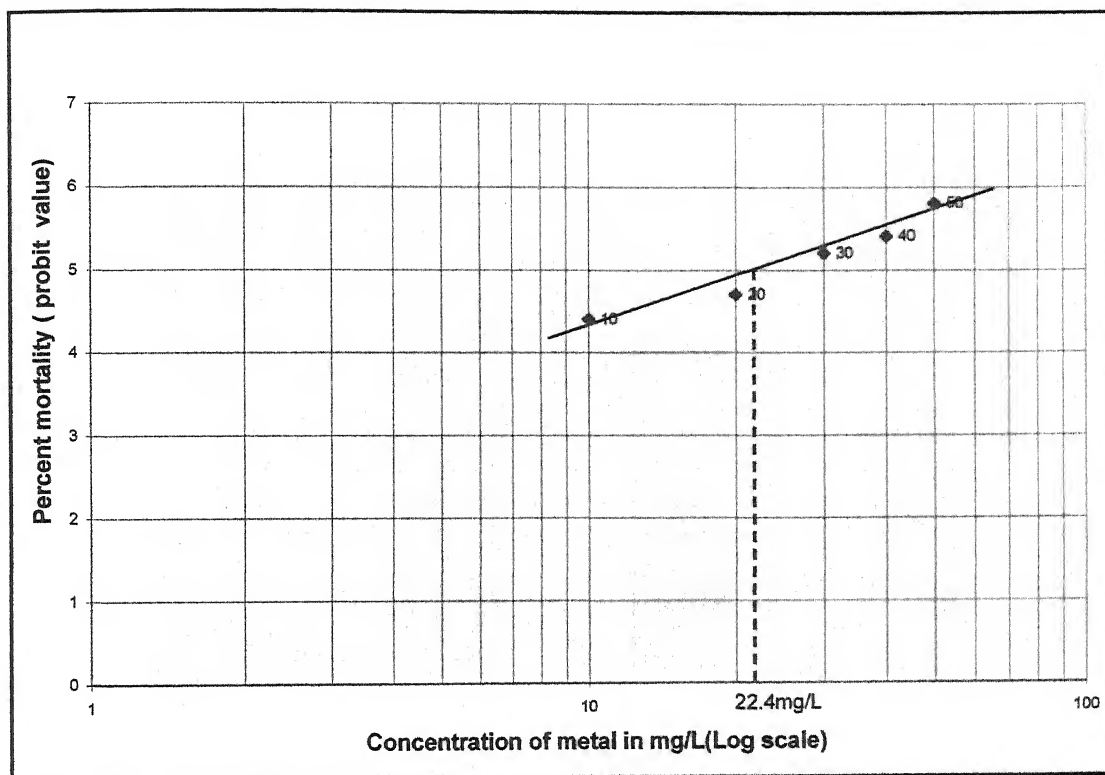
Determination of 48 hrs. median lethal concentration (48h LC 50)
of Copper sulphate to *H.fossilis* (Bloch) by probit analysis.

Fig-15



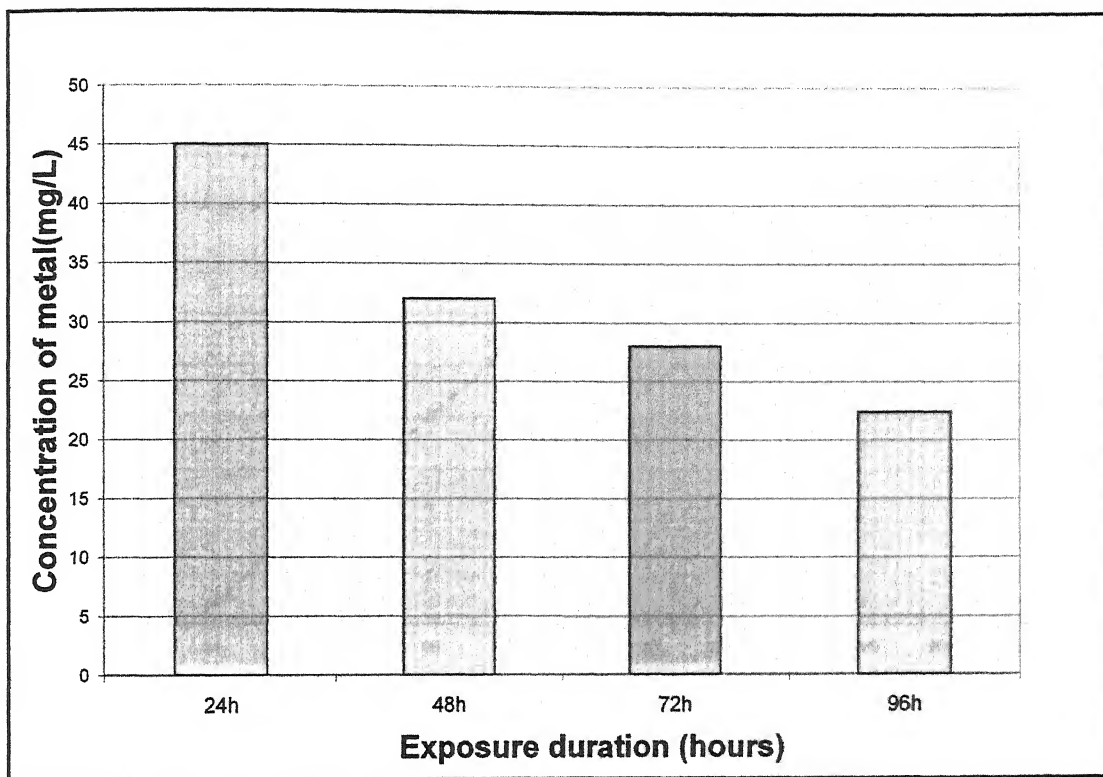
Determination of 72 hrs. median lethal concentration (72h LC 50)
of Copper sulphate to *H.fossilis* (Bloch) by probit analysis.

Fig-16



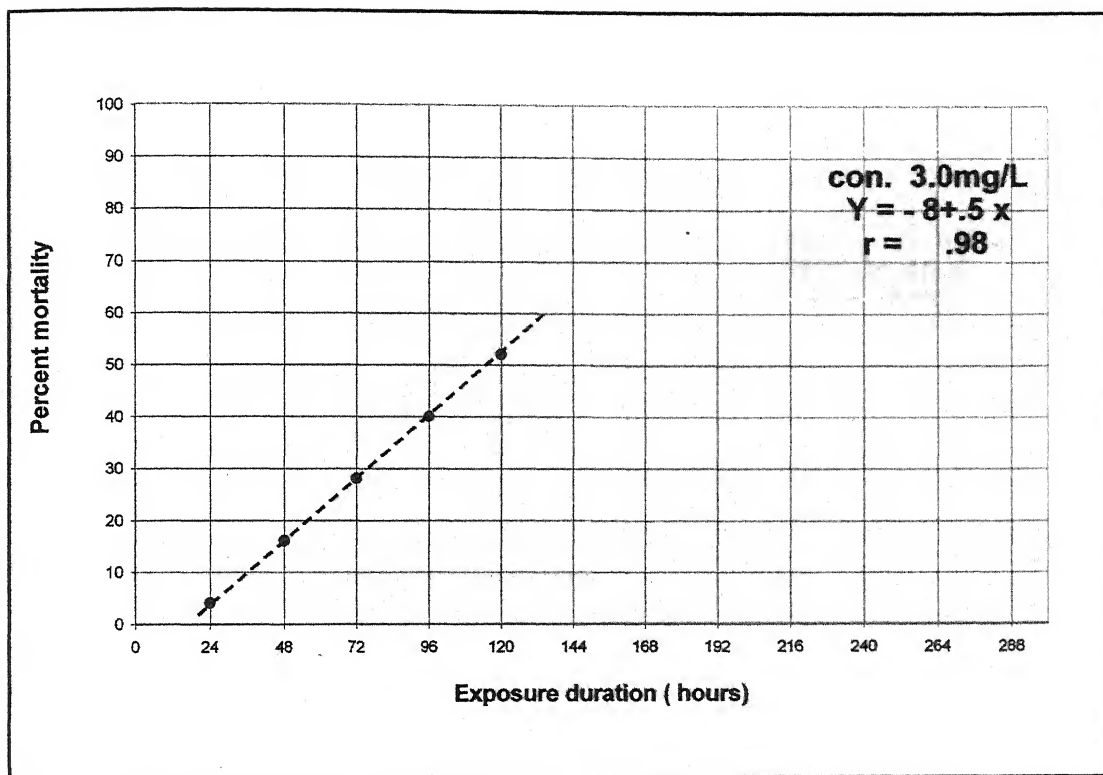
Determination of 96 hrs. median lethal concentration (96h LC 50)
of Copper sulphate to *H.fossilis* (Bloch) by probit analysis.

Fig-17



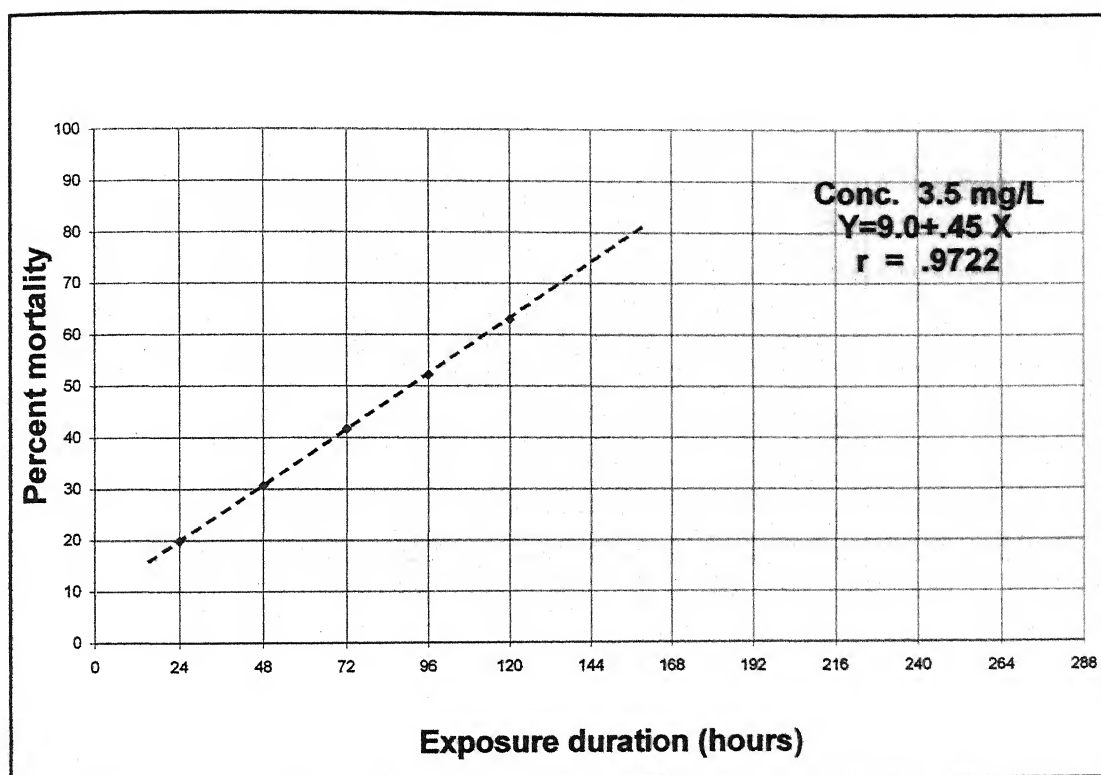
LC 50 values of Copper sulphate in different exposure period in
H.fossilis (Bloch)

Fig-18



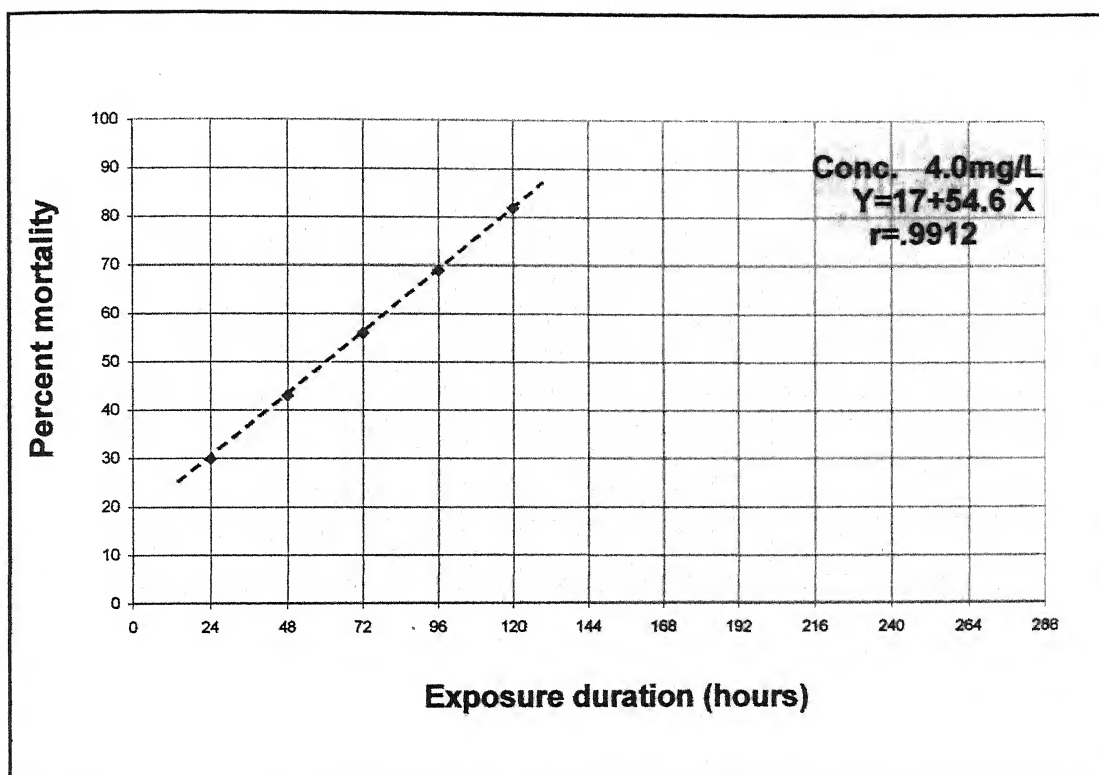
Regression line showing relationship between exposure duration and percentage of mortality in *C. carpio* (Linn.) exposed to 3.0 mg/L concentration of Cadmium chloride.

Fig-19



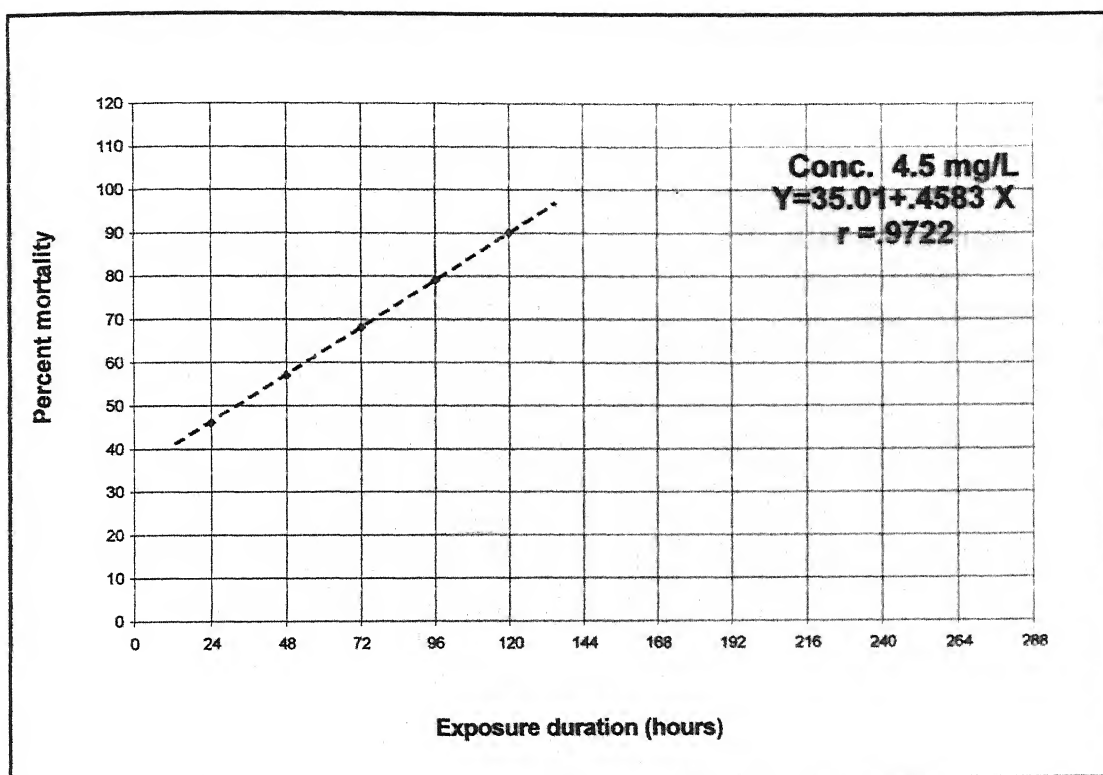
Regression line showing relationship between exposure duration and percentage of mortality in *C. carpio*(Linn.) exposed to 3.5 mg/L concentration of cadmium chloride.

Fig-20



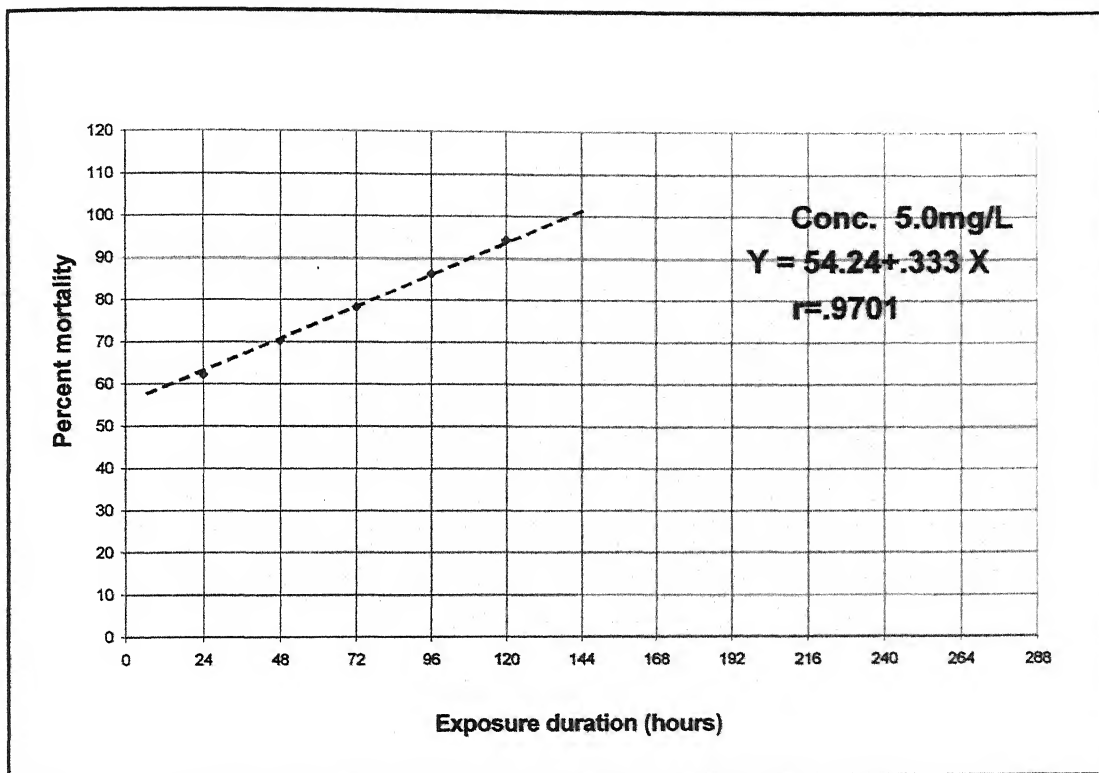
Regression line showing relationship between exposure duration and percentage of mortality in *C. carpio* (Linn.) exposed to 4.0 mg/L concentration of cadmium chloride.

Fig-21



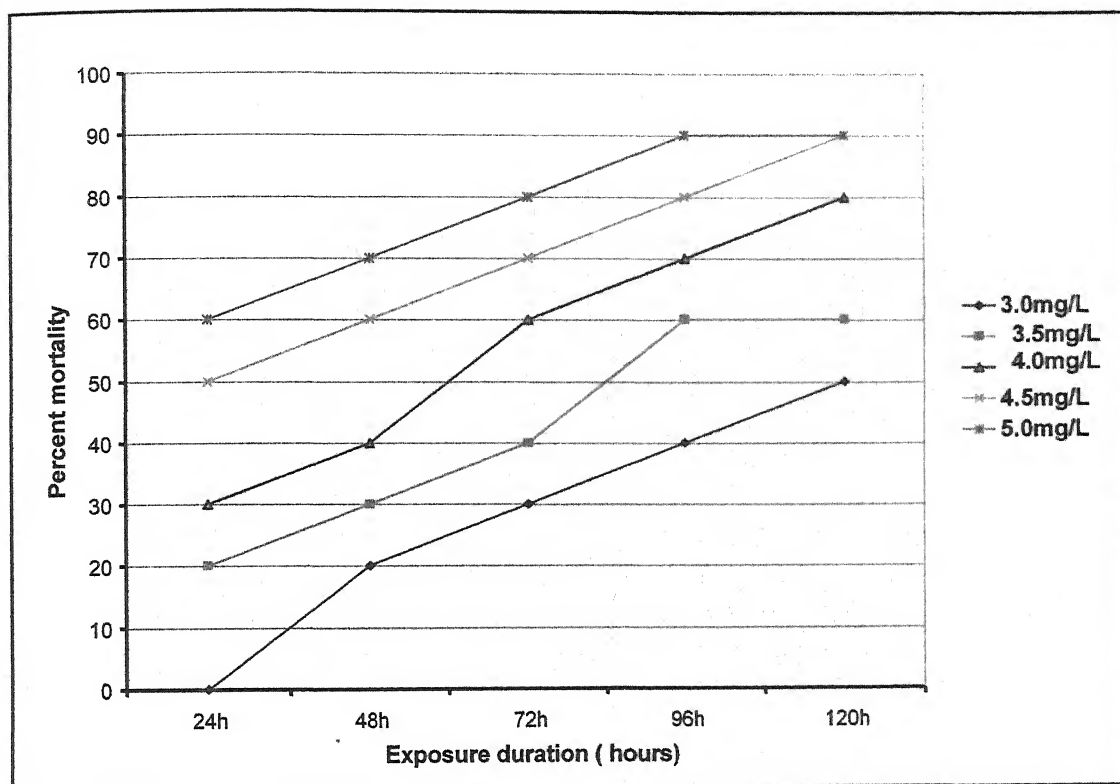
Regression line showing relationship between exposure duration and percentage of mortality in *C. carpio* (Linn.) exposed to 4.5 mg/L concentration of Cadmium chloride.

Fig-22



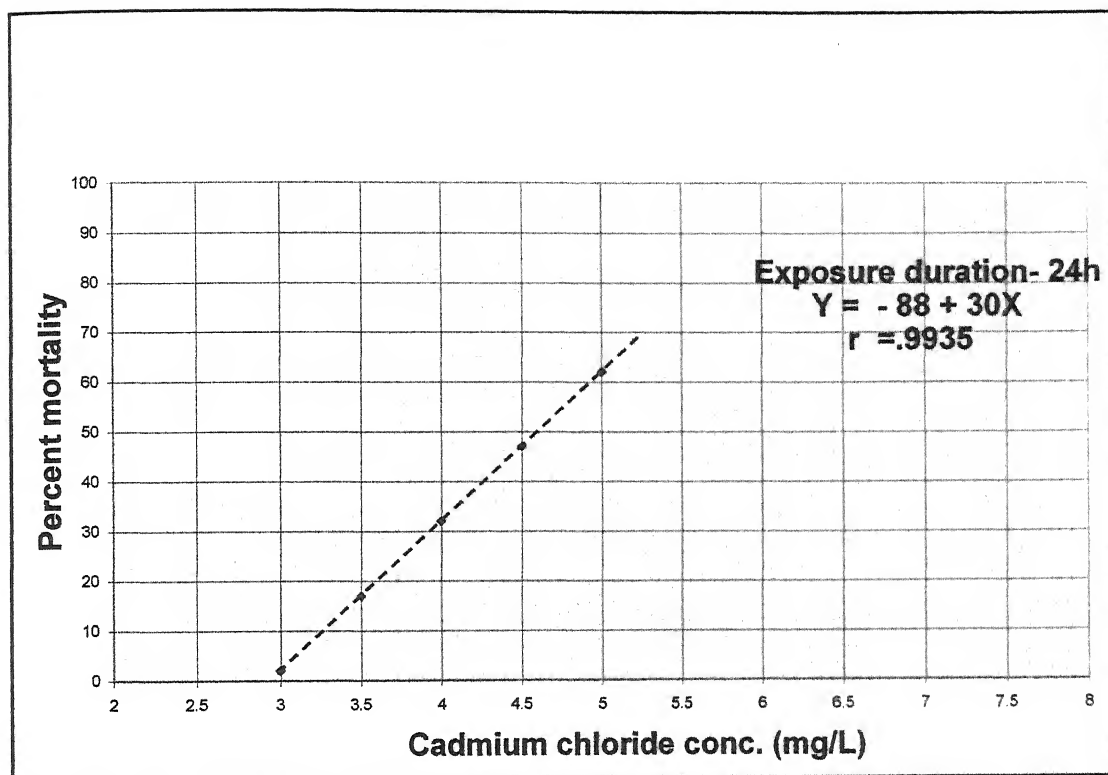
Regression line showing relationship between exposure duration and percentage of mortality in *C. carpio* (Linn.) exposed to 5.0 mg/L concentration of Cadmium chloride.

Fig-23



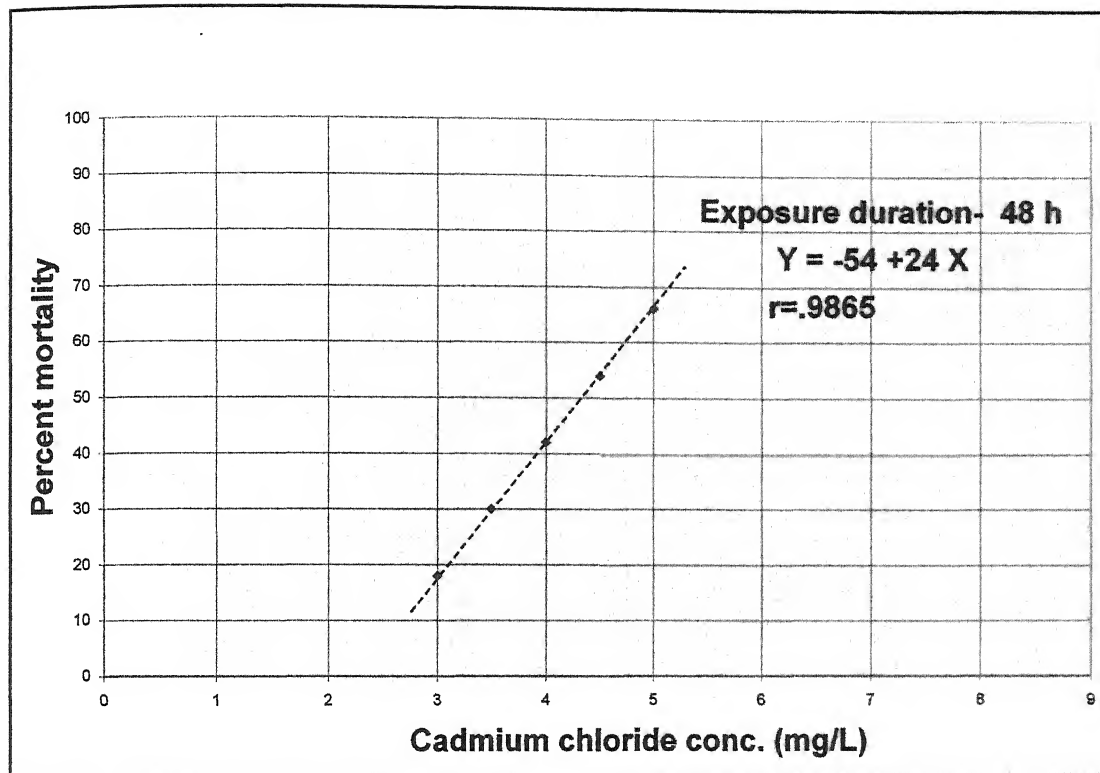
Time influenced percentage of mortality of *C. carpio* (Linn.) in different concentrations of Cadmium chloride.

Fig-24



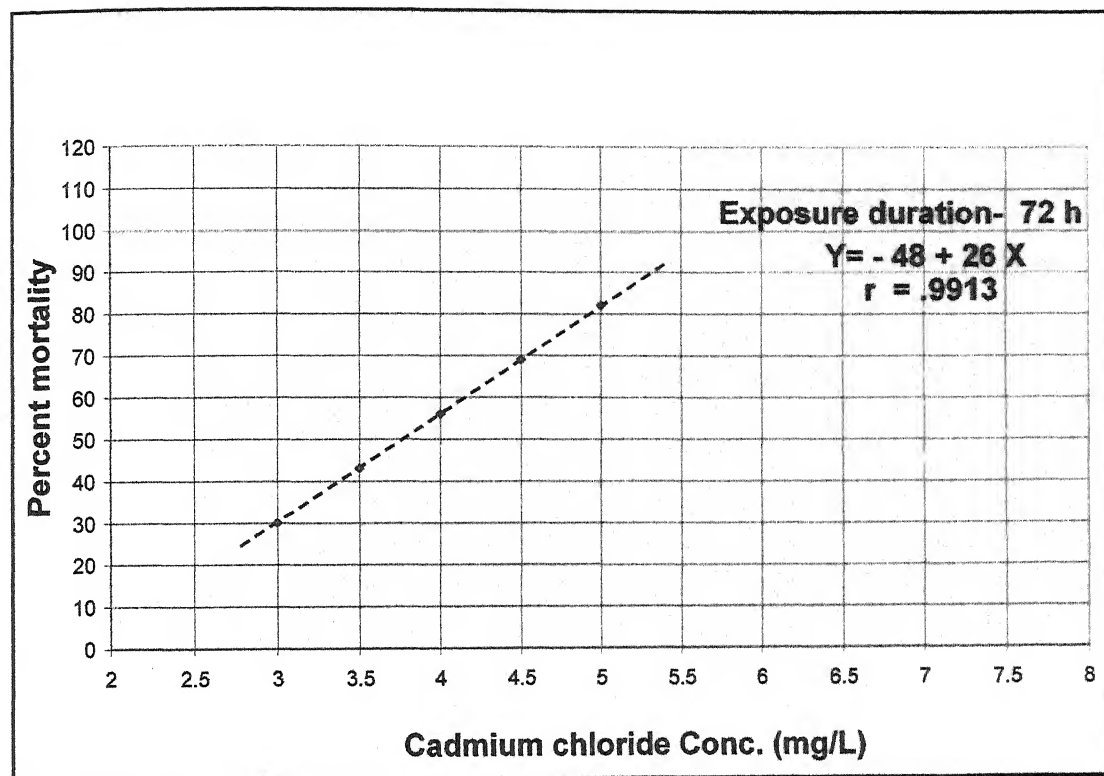
Regression line showing relationship between different concentrations of Cadmium chloride and percentage of mortality of *C. carpio* (Linn) in 24 h exposure duration .

Fig-25



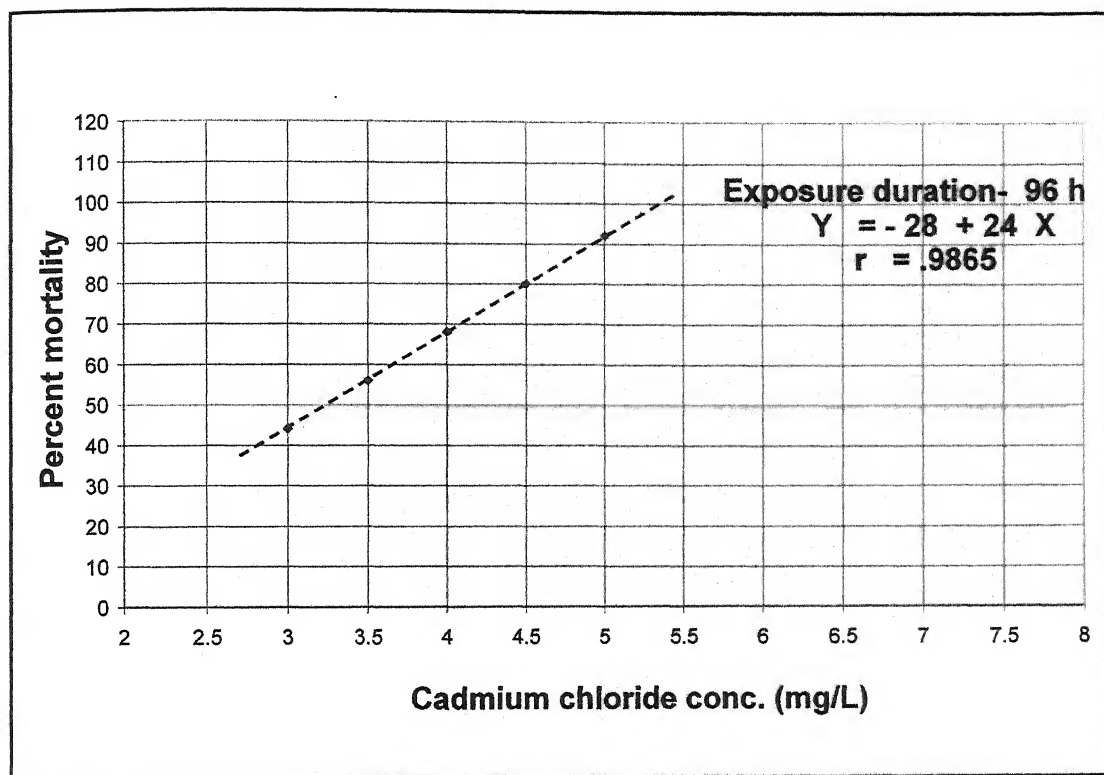
Regression line showing relationship between different Concentrations of Cadmium chloride and percentage of mortality of *C. carpio* (Linn.) in 48 h exposure duration.

Fig-26



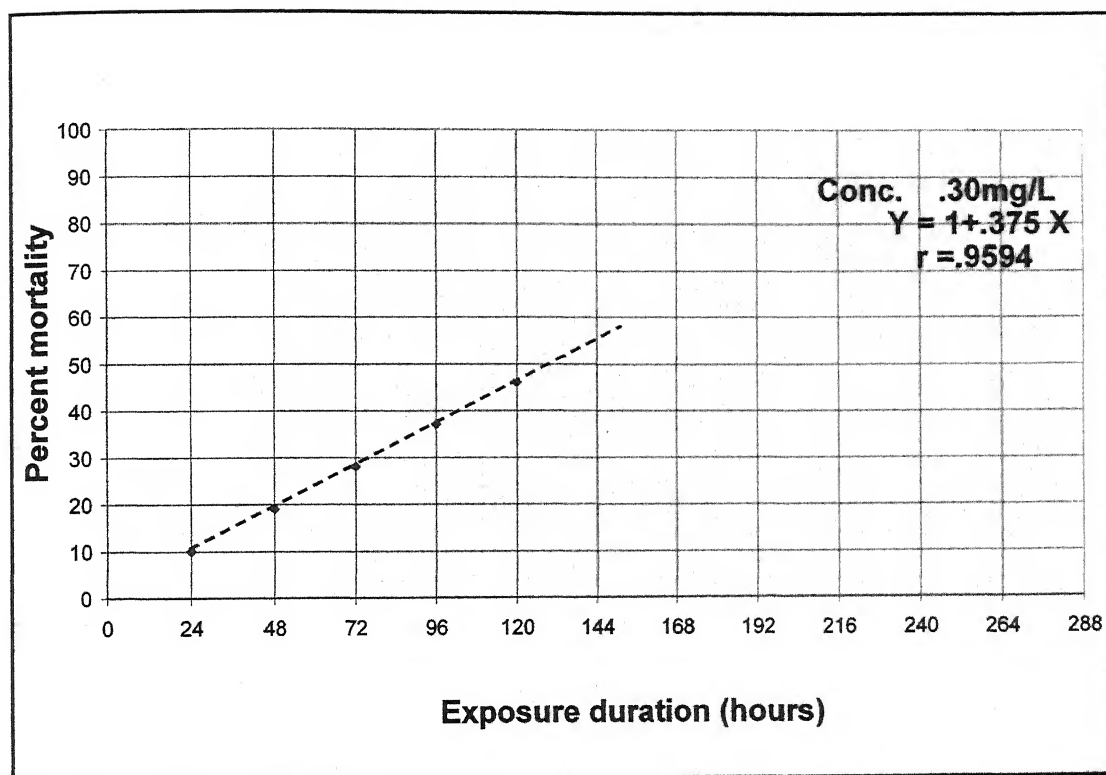
Regression line showing relationship between different concentration of Cadmium chloride and percentage of mortality of *C. carpio* (Linn.) in 72h exposure duration.

Fig-27



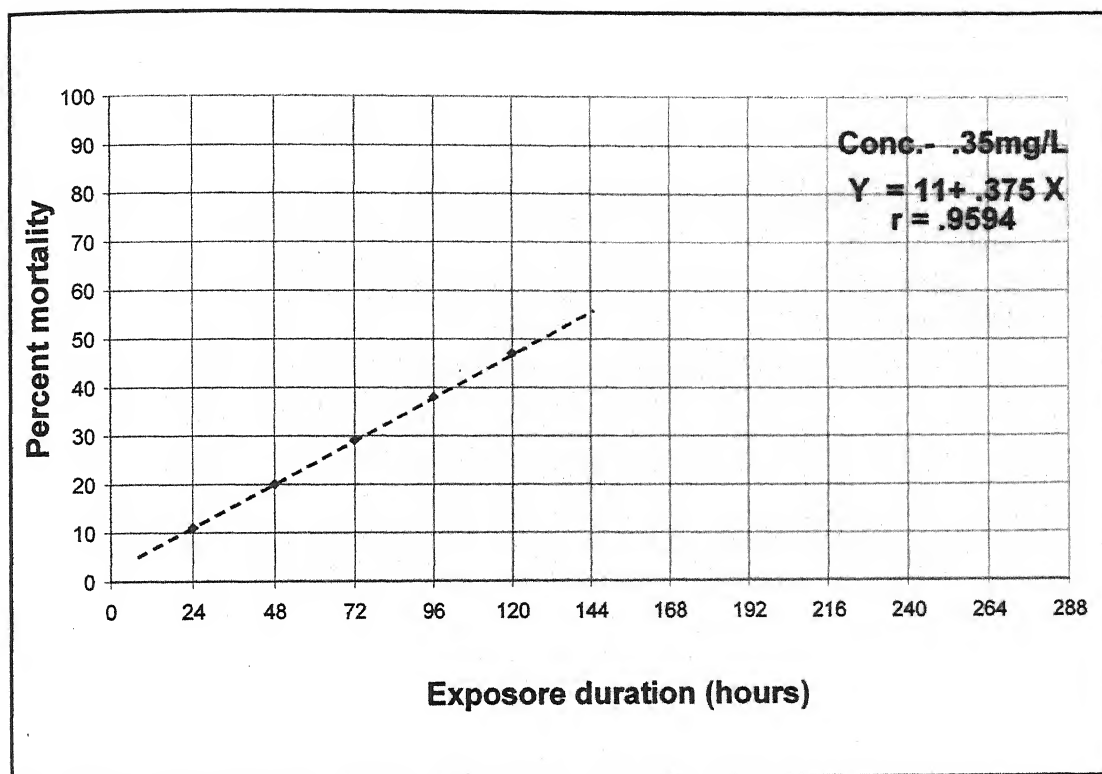
Regression line showing relationship between different concentrations of Cadmium chloride and percentage of mortality of *C. carpio* (Linn.) in 96h exposure duration.

Fig-28



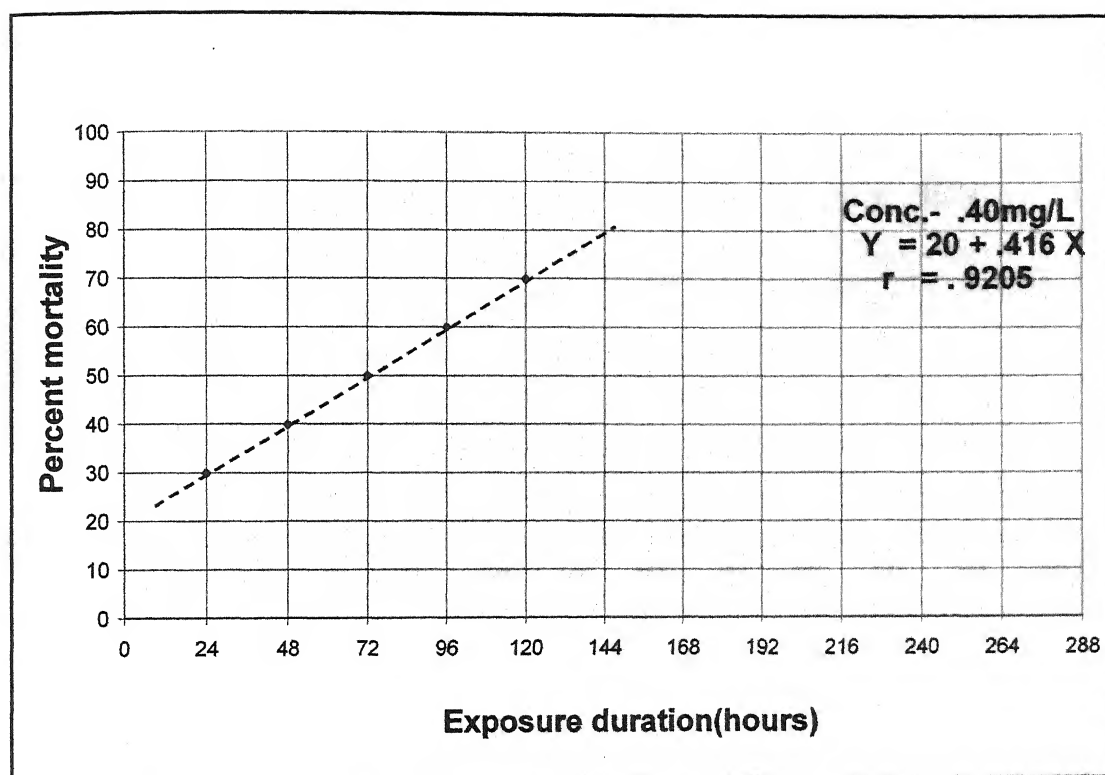
Regression line showing relationship between exposure duration and percentage of mortality in *C. carpio*(Linn.) exposed to .30 mg/L concentration of Mercuric chloride.

Fig-29



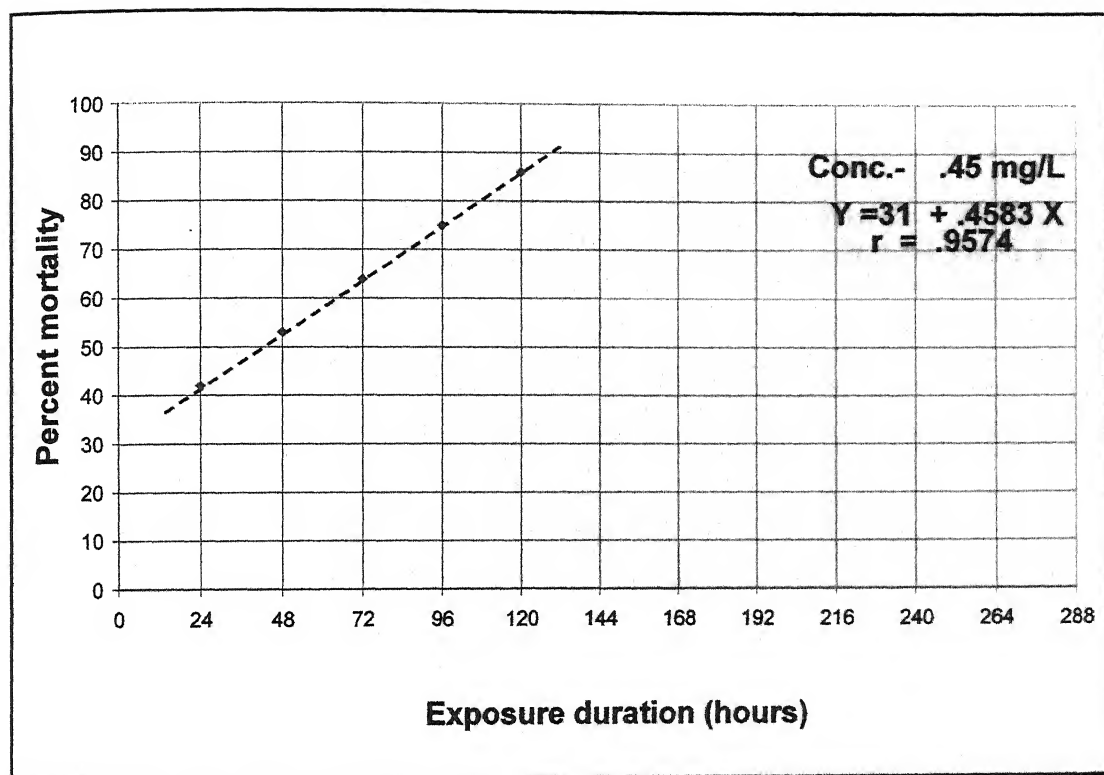
Regression line showing relationship between exposure duration and percentage of mortality in *C. carpio* (Linn.) exposed to .35 mg/L concentration of Mercuric chloride.

Fig-30



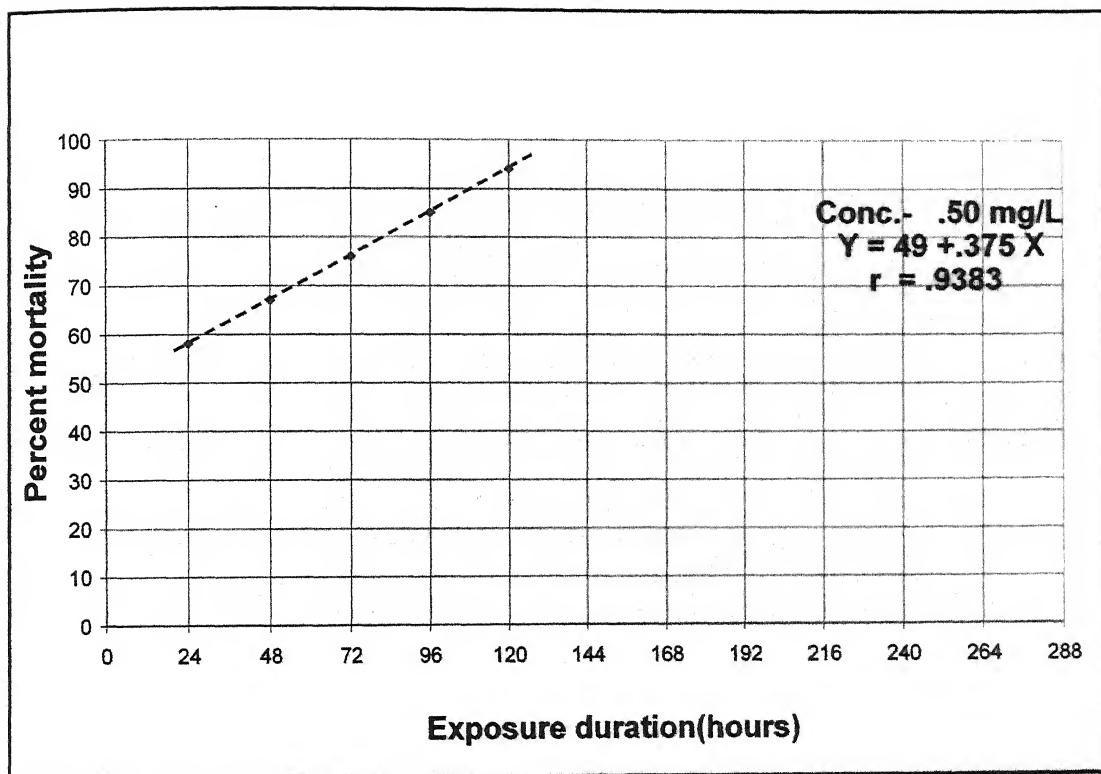
Regression line showing relationship between exposure duration and percentage of mortality in *C. carpio* (Linn.) exposed to 40mg/L concentration of Mercuric chloride.

Fig-31



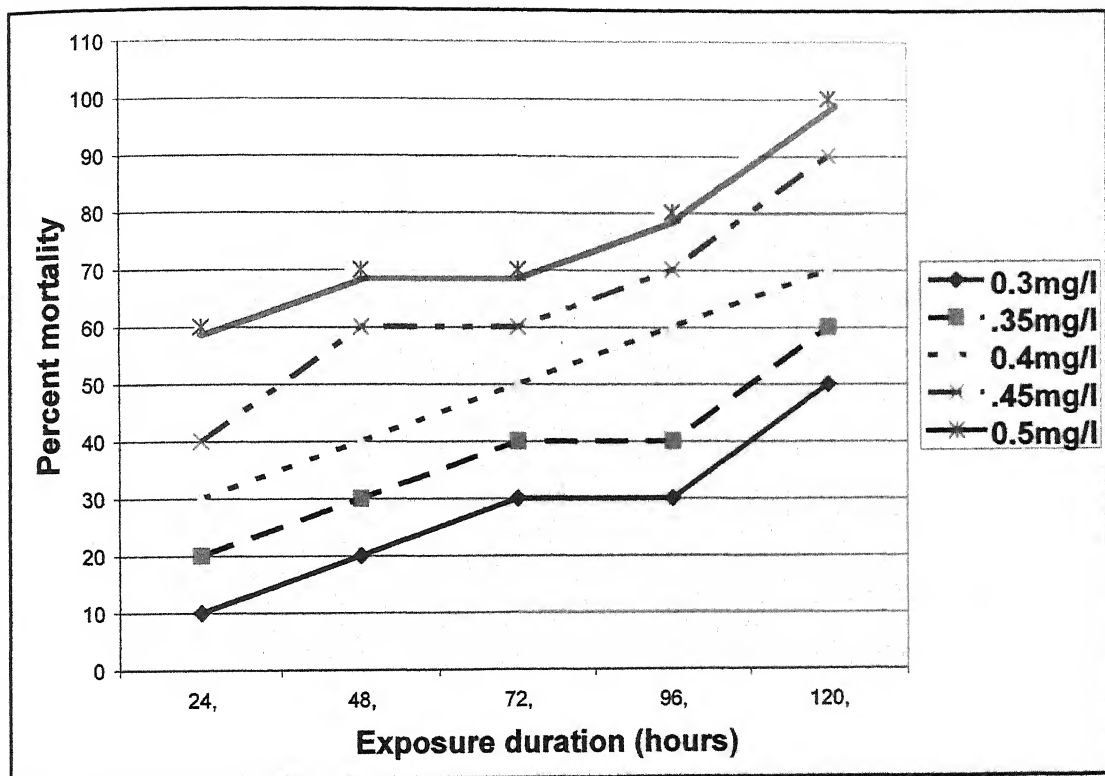
Regression line showing relationship between exposure duration and percentage of mortality in *C. carpio*(Linn.) exposed to .45 mg/L concentration of Mercuric chloride.

Fig-32



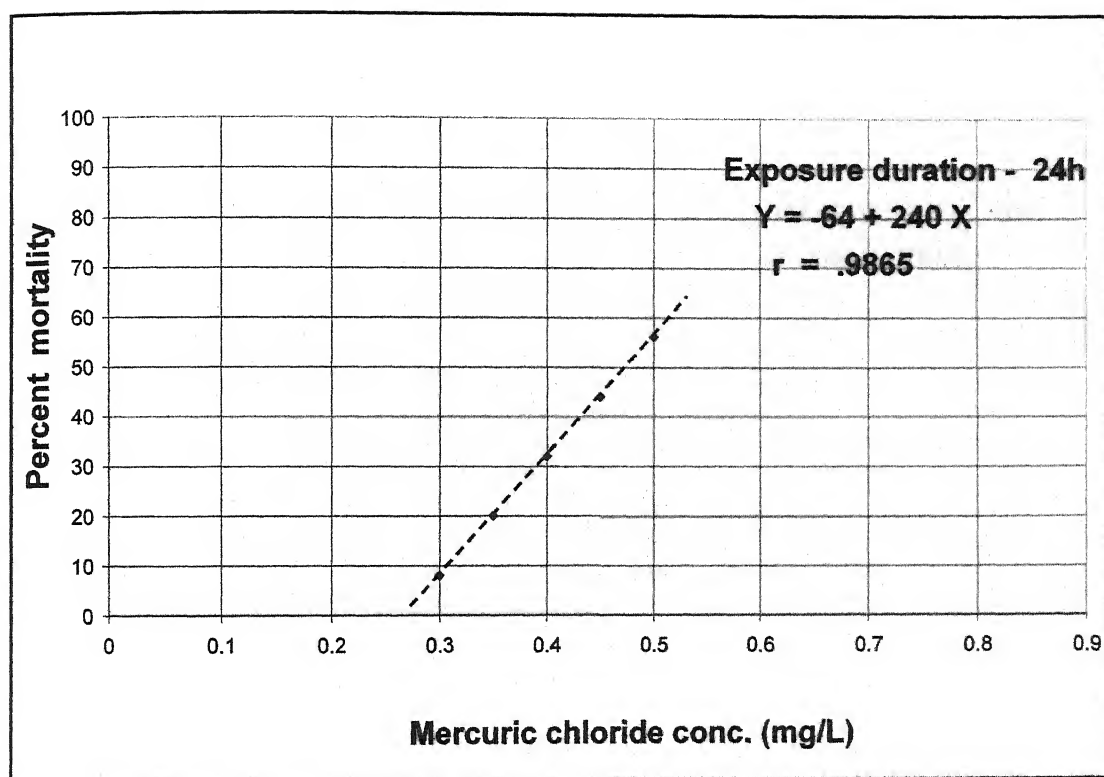
Regression line showing relationship between exposure duration and percentage of mortality in *C. carpio* (Linn.) exposed to .50 mg/L concentration of Mercuric chloride.

Fig-33



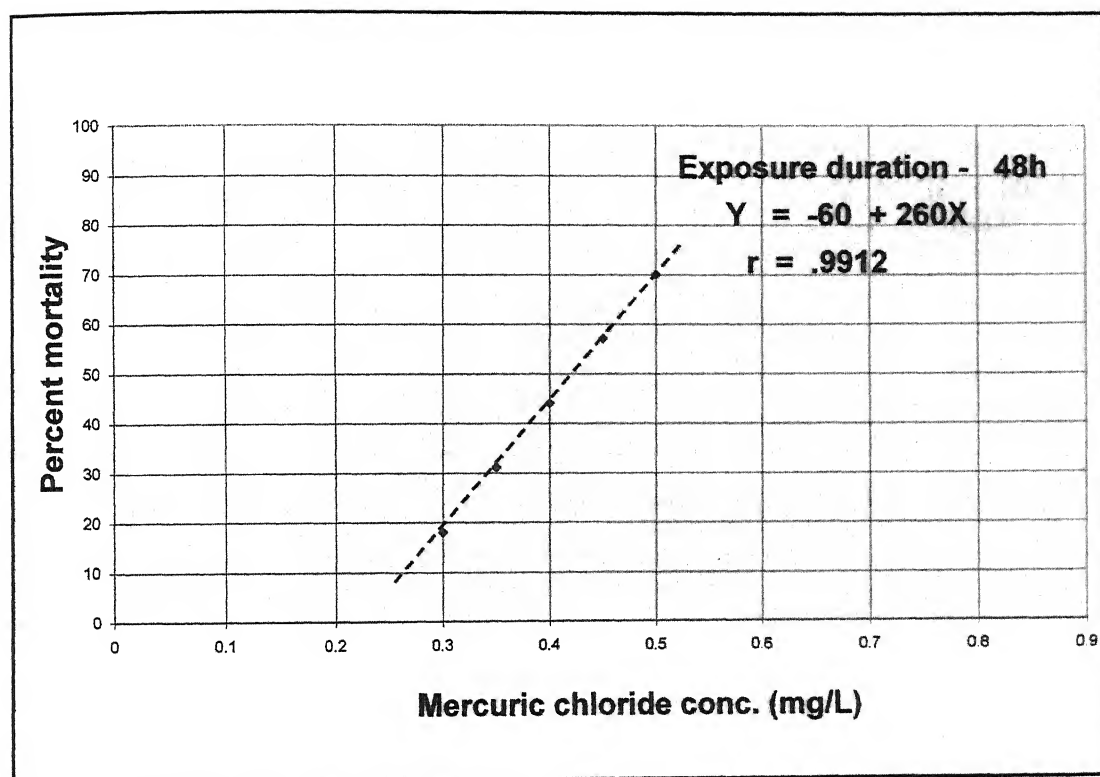
Time influenced percentage of mortality of *C. carpio* (Linn.) in different concentrations of Mercuric chloride.

Fig-34



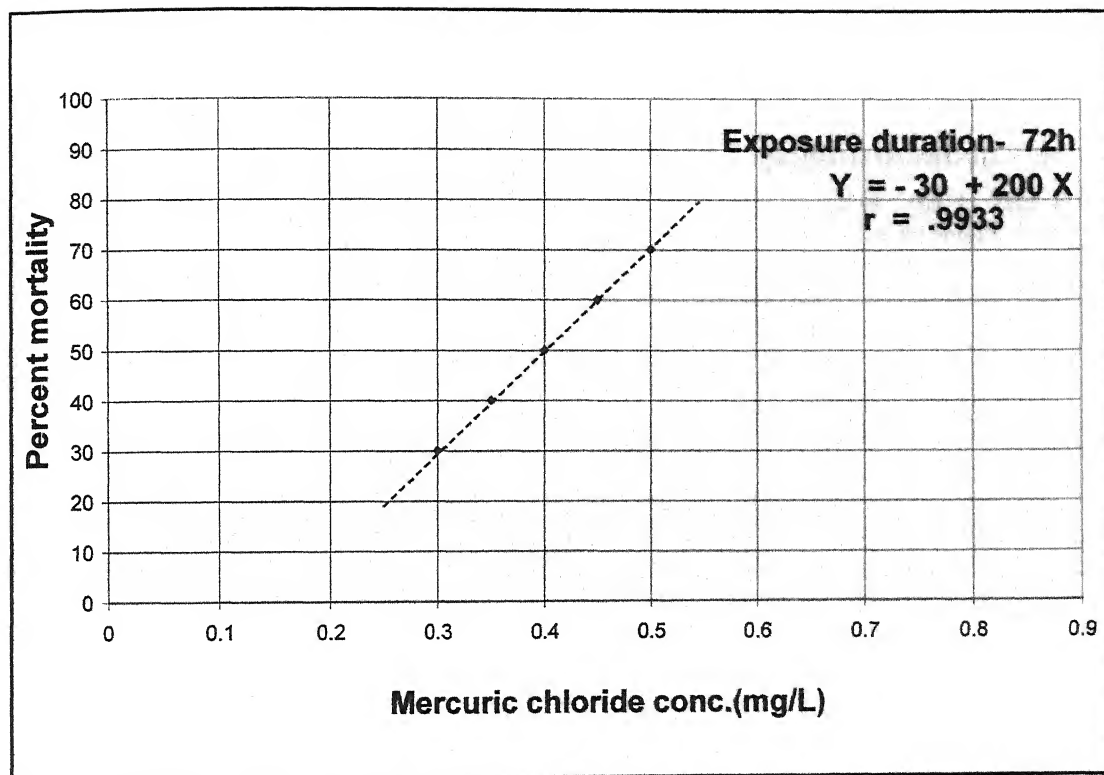
Regression line showing relationship between different concentrations of Mercuric chloride and percentage of mortality of *C. carpio* (Linn.) in 24 h exposure duration .

Fig-35



Regression line showing relationship between different concentrations of Mercuric chloride and percentage of mortality of *C. carpio* (Linn.) in 48 h exposure duration .

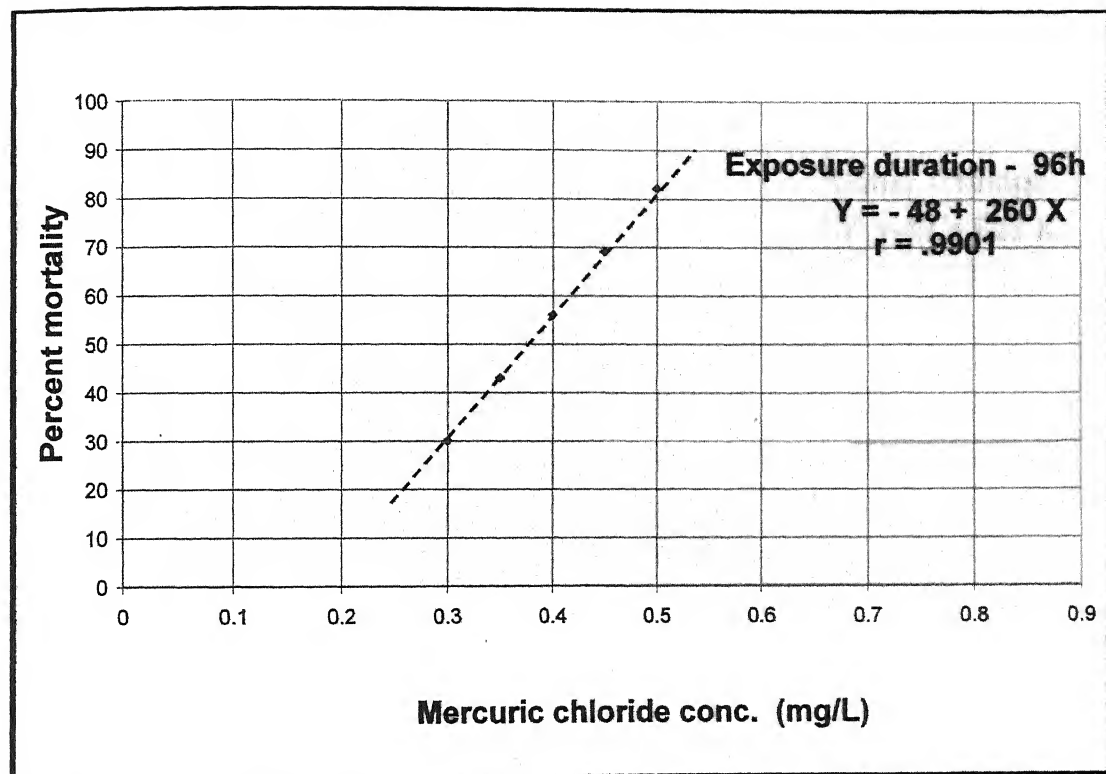
Fig-36



Regression line showing relationship between different concentrations of Mercuric chloride and percentage of mortality of *C. carpio* (Linn.) in 72 h exposure duration .

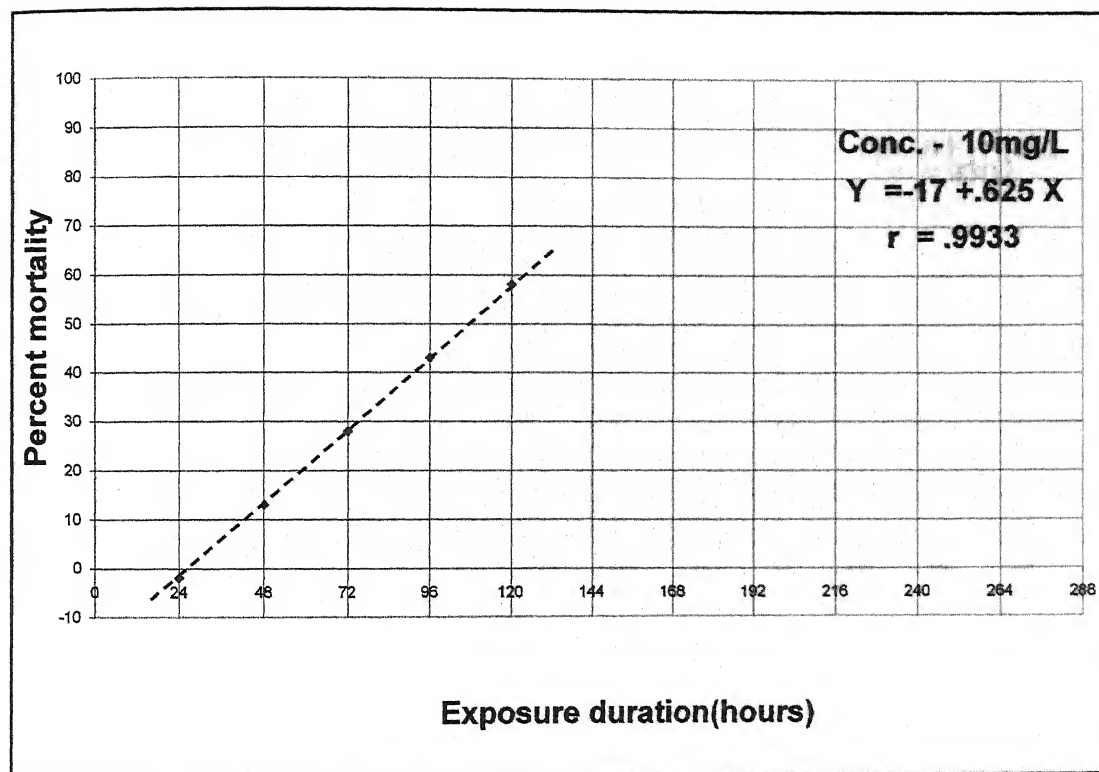
Fig-37

*



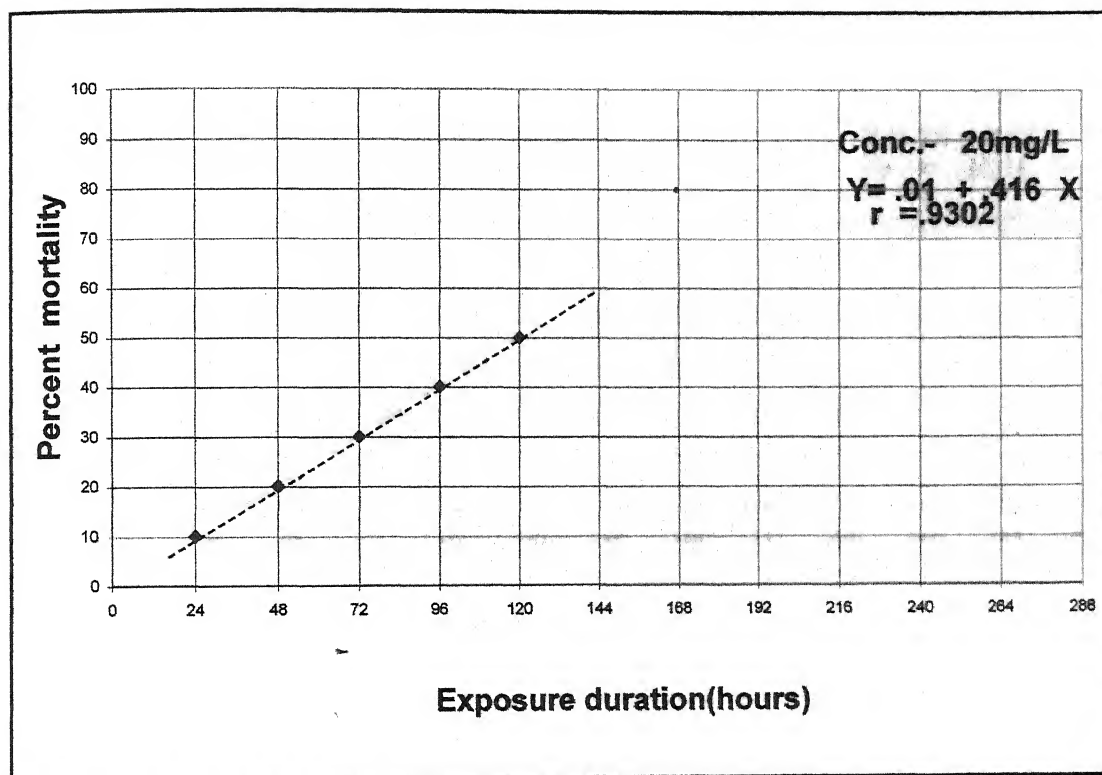
Regression line showing relationship between different concentrations of Mercuric chloride and percentage of mortality of *C. carpio* (Linn.) in 96h exposure duration .

Fig-38



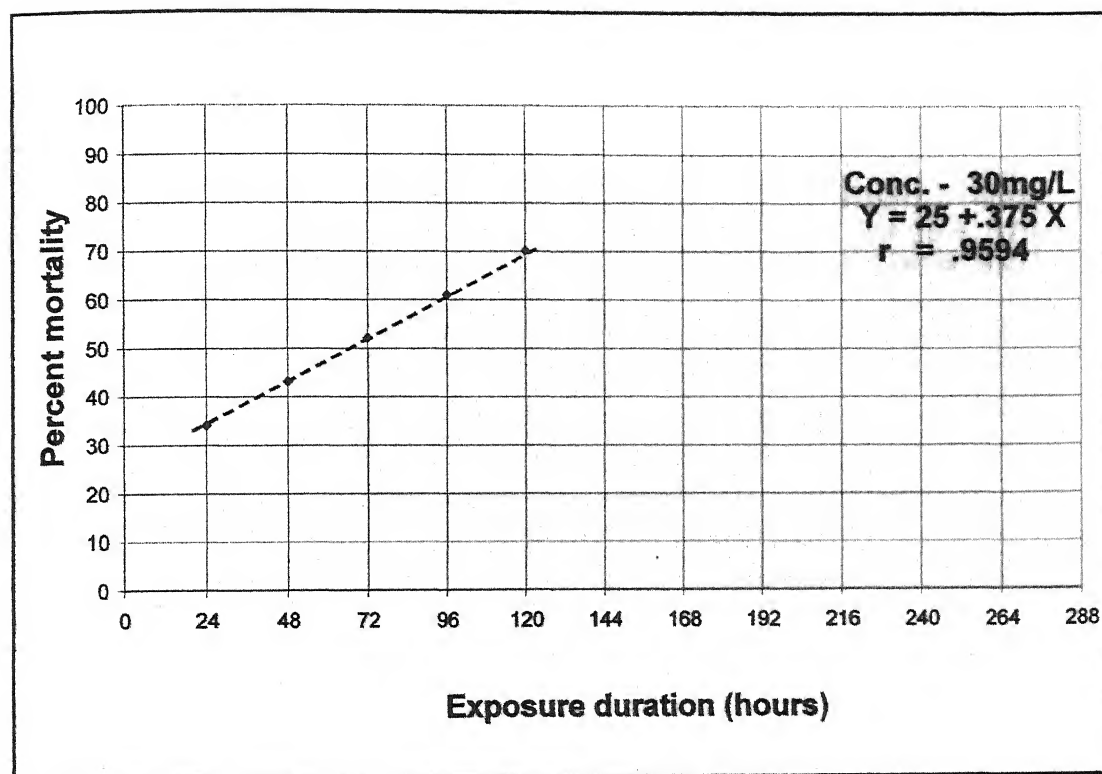
Regression line showing relationship between exposure duration and percentage of mortality in *H.fossilis*(Bloch) exposed to 10 mg/L concentration of Copper sulphate.

Fig-39



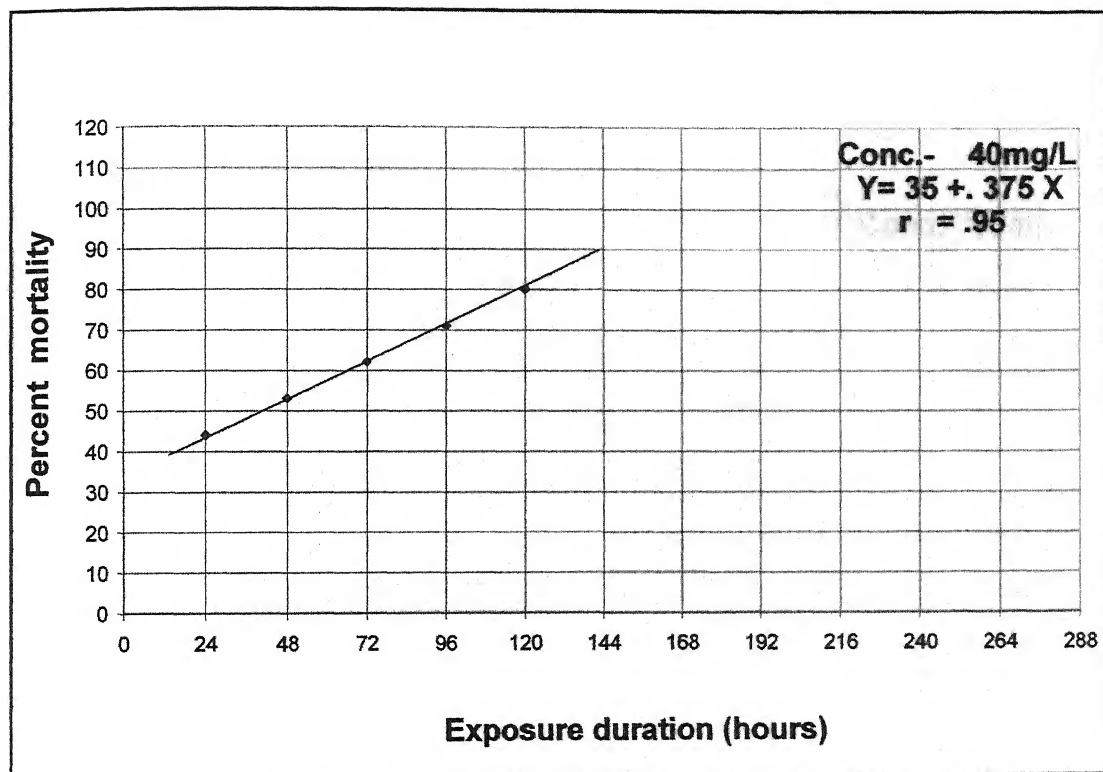
Regression line showing relationship between exposure duration and percentage of mortality in *H.fossilis* (Bloch) exposed to 20 mg/L concentration of Copper sulphate.

Fig-40



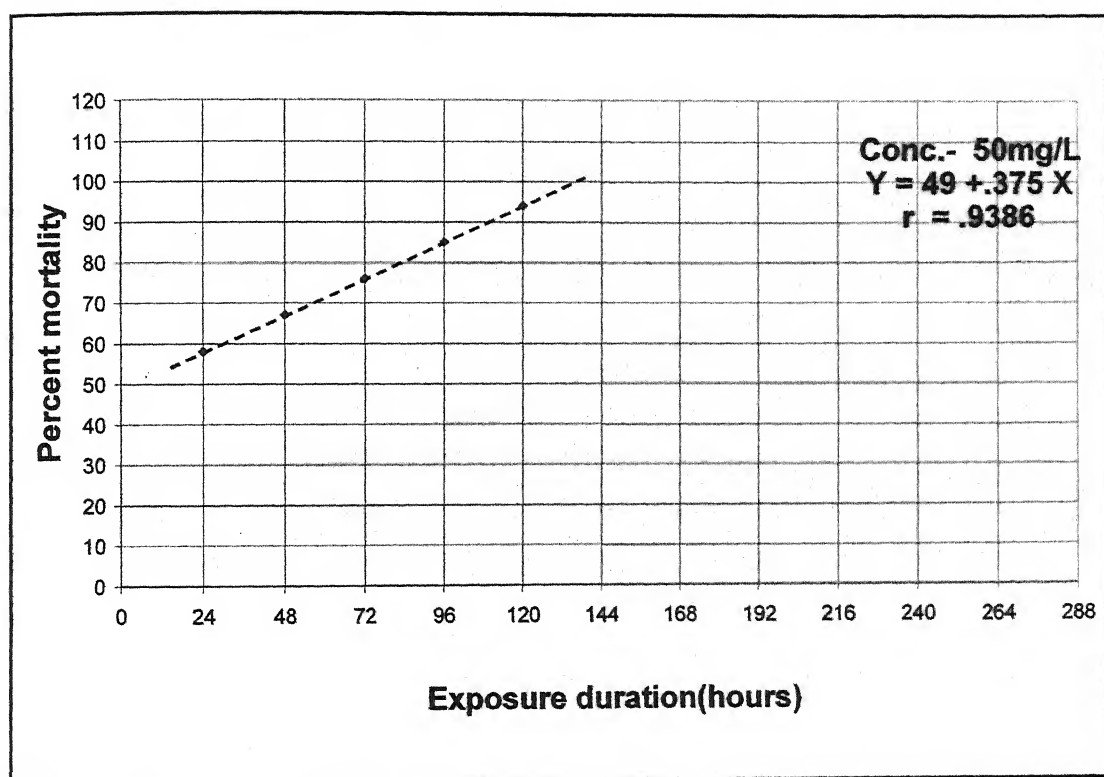
Regression line showing relationship between exposure duration and percentage of mortality in *H.fossilis*(Bloch) exposed to 30 mg/L concentration of Copper sulphate.

Fig-41



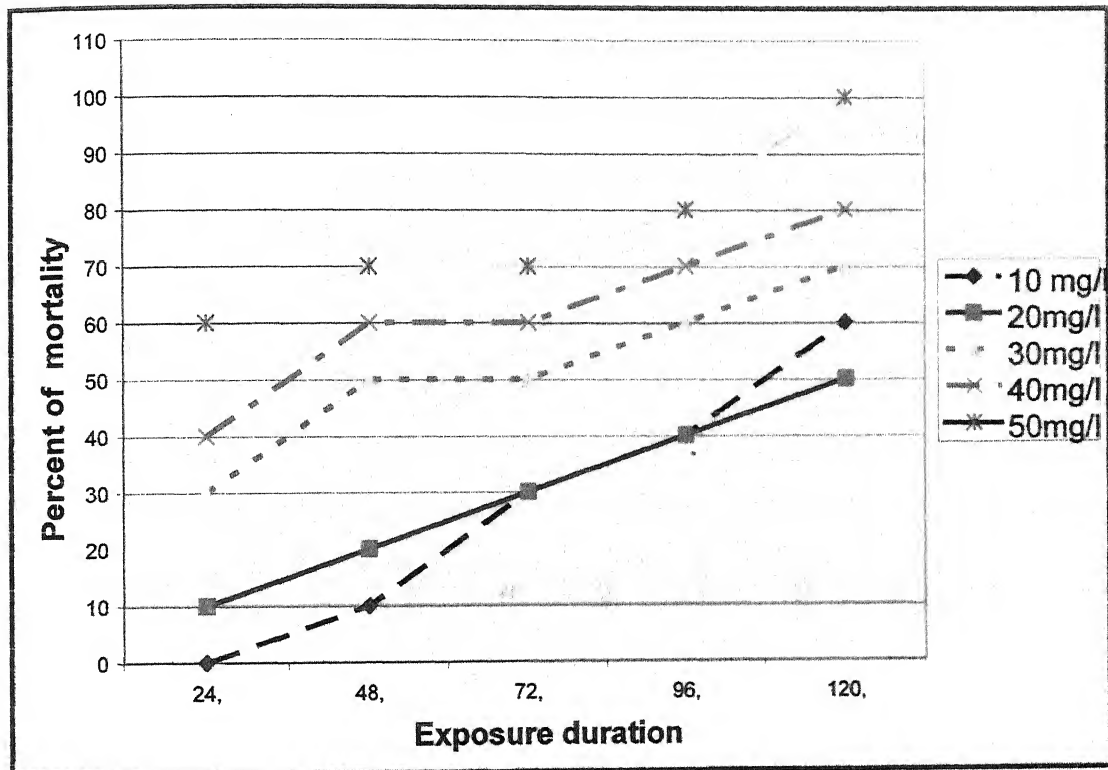
Regression line showing relationship between exposure duration and percentage of mortality in *H.fossilis*(Bloch) exposed to 40 mg/L concentration of Copper sulphate.

Fig-42



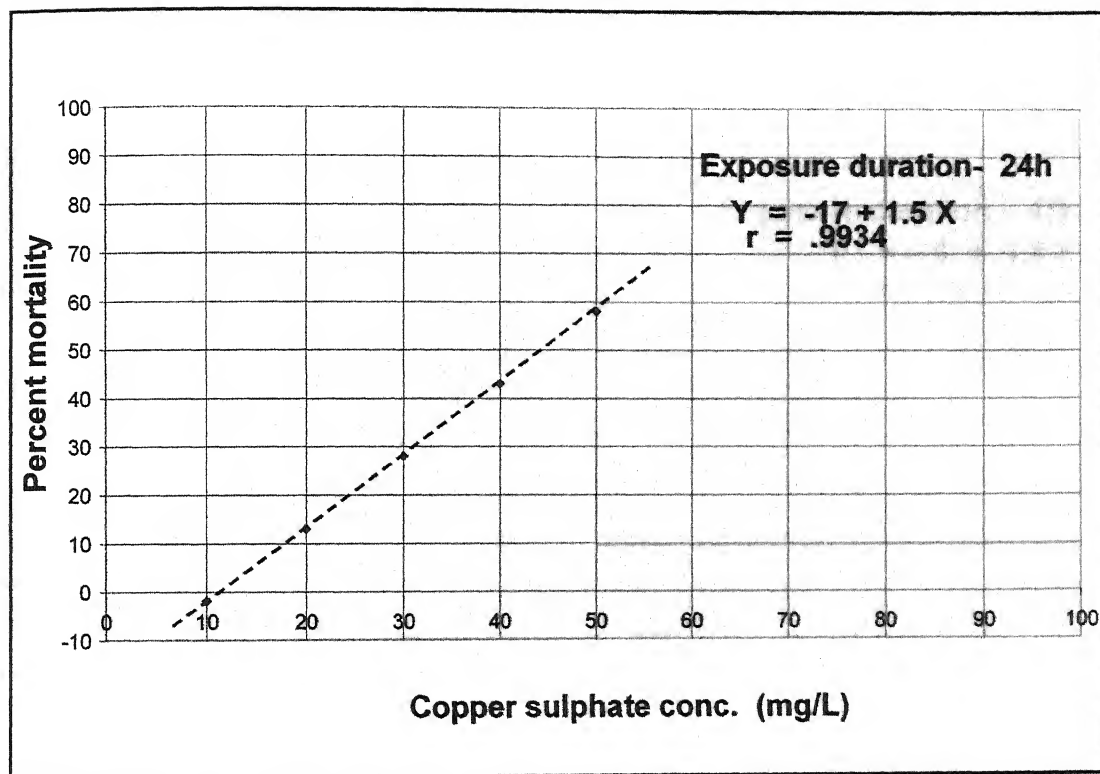
Regression line showing relationship between exposure duration and percentage of mortality in *H.fossilis* (Bloch) exposed to 50 mg/L concentration of Copper sulphate.

Fig-43



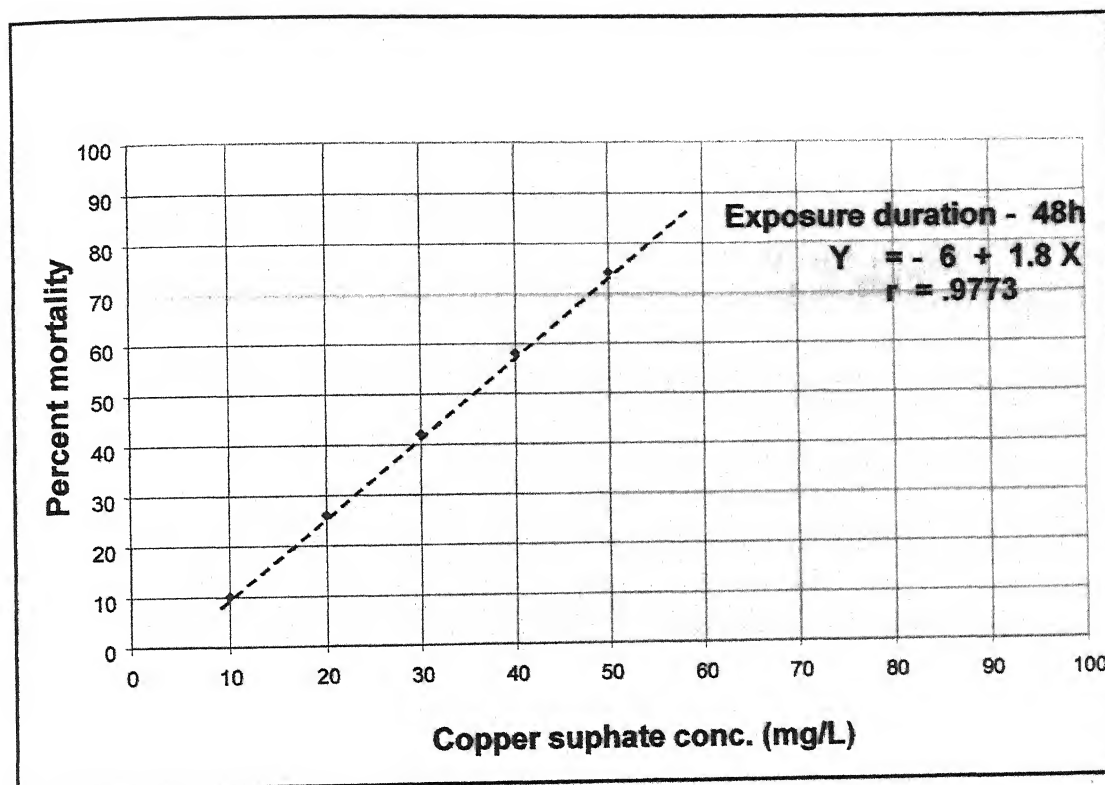
Time influenced percentage of mortality of *H. fossilis* (Bloch) in different concentrations of Copper sulphate.

Fig-44



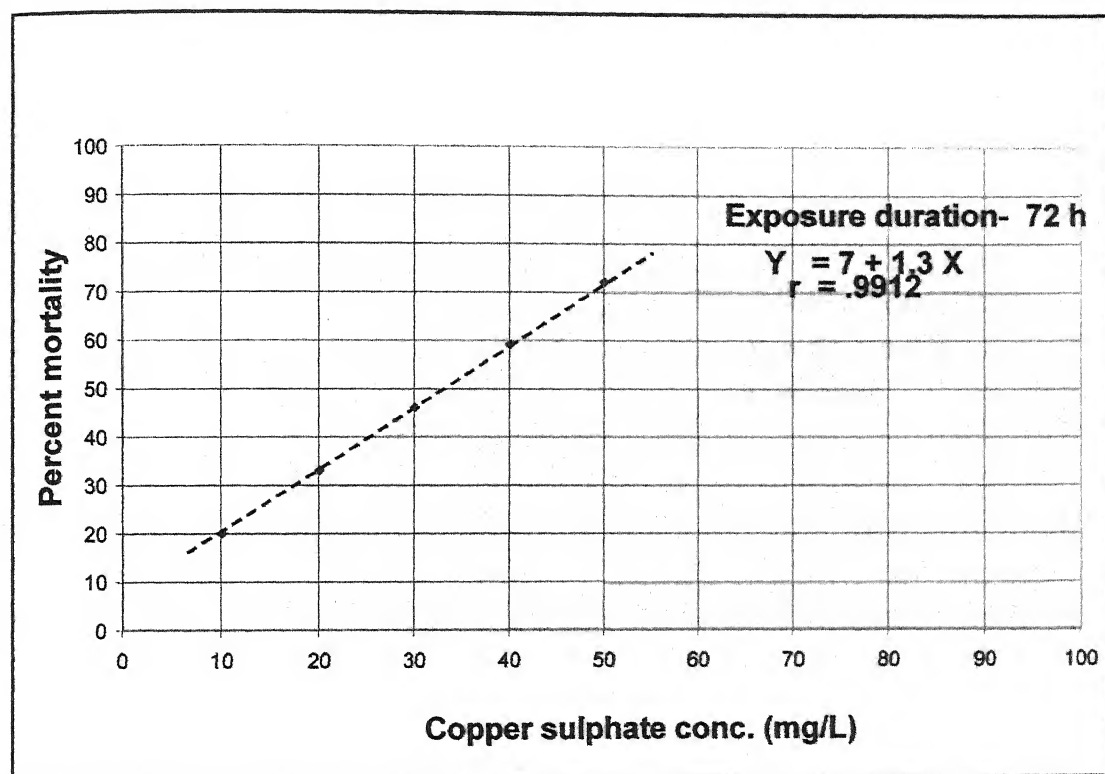
Regression line showing relationship between different concentrations of Copper sulphate and percentage of mortality of *H.fossilis* (Bloch) in 24h exposure duration .

Fig-45



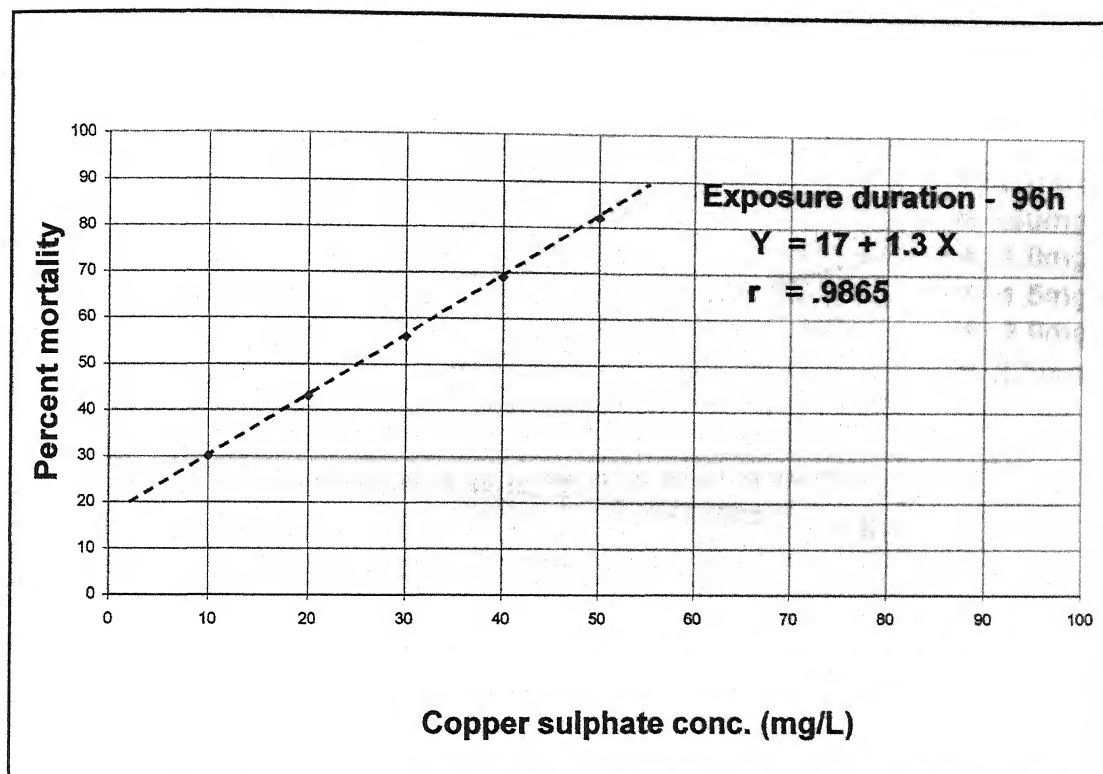
Regression line showing relationship between different concentrations of Copper sulphate and percentage of mortality of *H.fossilis* (Bloch) in 48 h exposure duration .

Fig-46



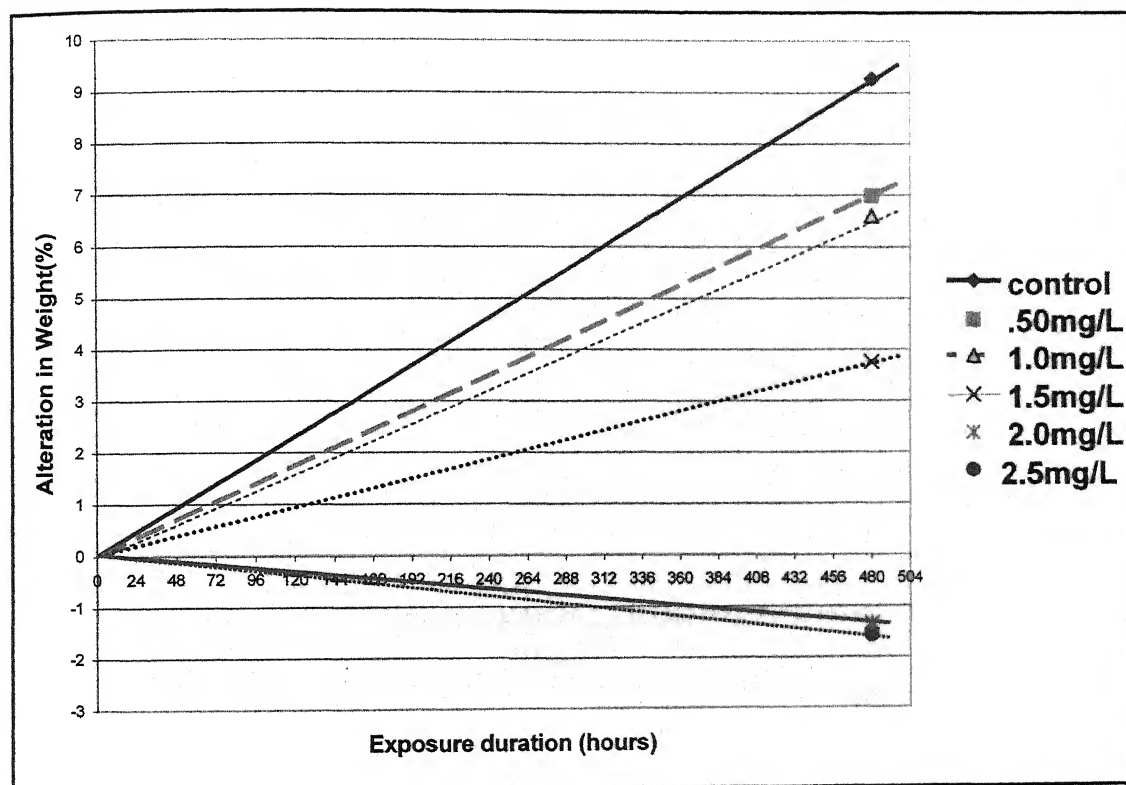
Regression line showing relationship between different concentrations of Copper sulphate and percentage of mortality of *H.fossilis* (Bloch) in 72 h exposure duration .

Fig-47



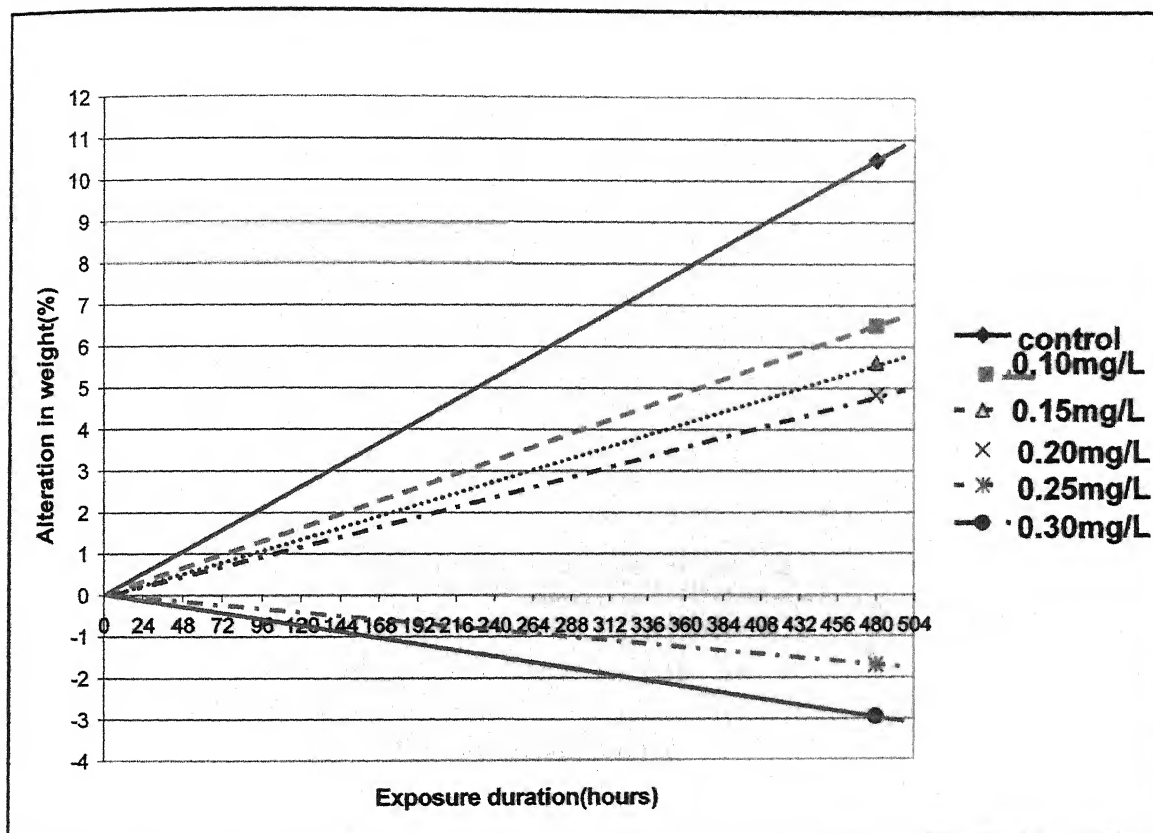
Regression line showing relationship between different concentrations of Copper sulphate and percentage of mortality of *H.fossilis*(Bloch) in 96h exposure duration .

Fig-48



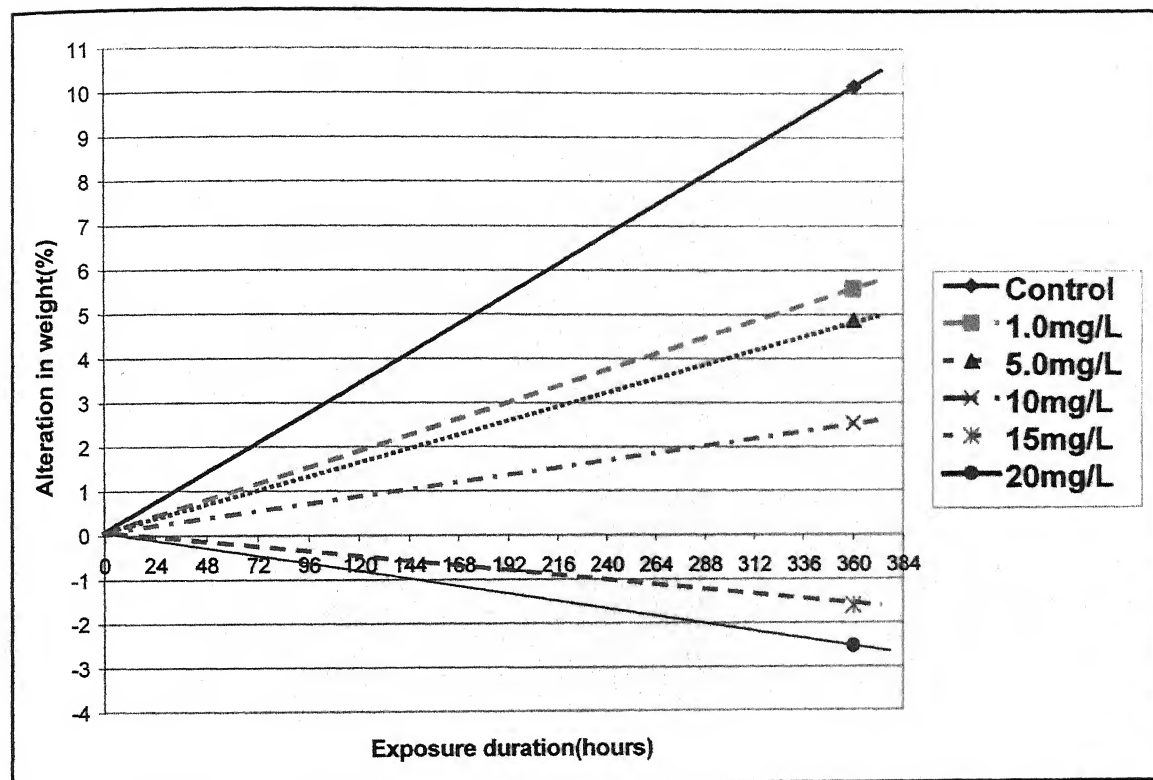
Alteration in growth (weight) of *C. carpio* (Linn) in different concentrations of Cadmium chloride (mg/L) in 480 h exposure duration.

Fig-49



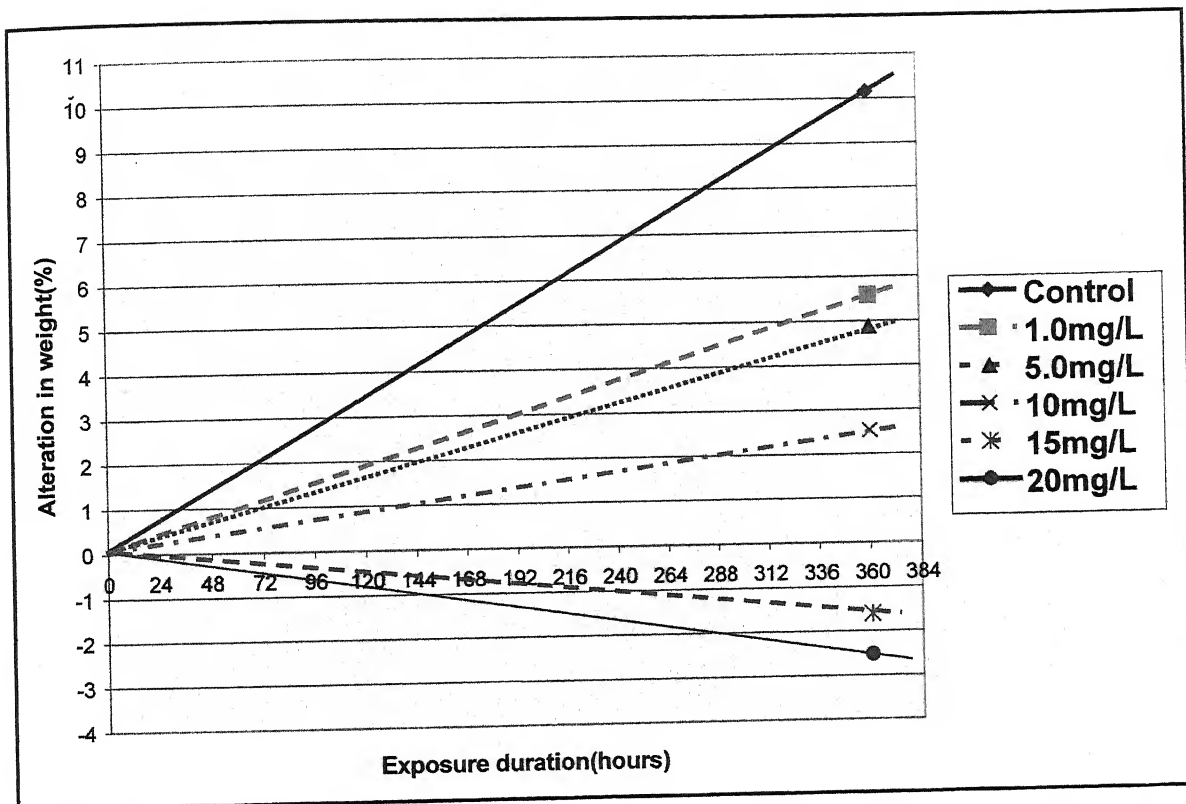
Alteration in growth (weight) of *C. carpio* (Linn) in different concentrations of Mercuric chloride (mg/L) in 480 h exposure duration.

Fig-50



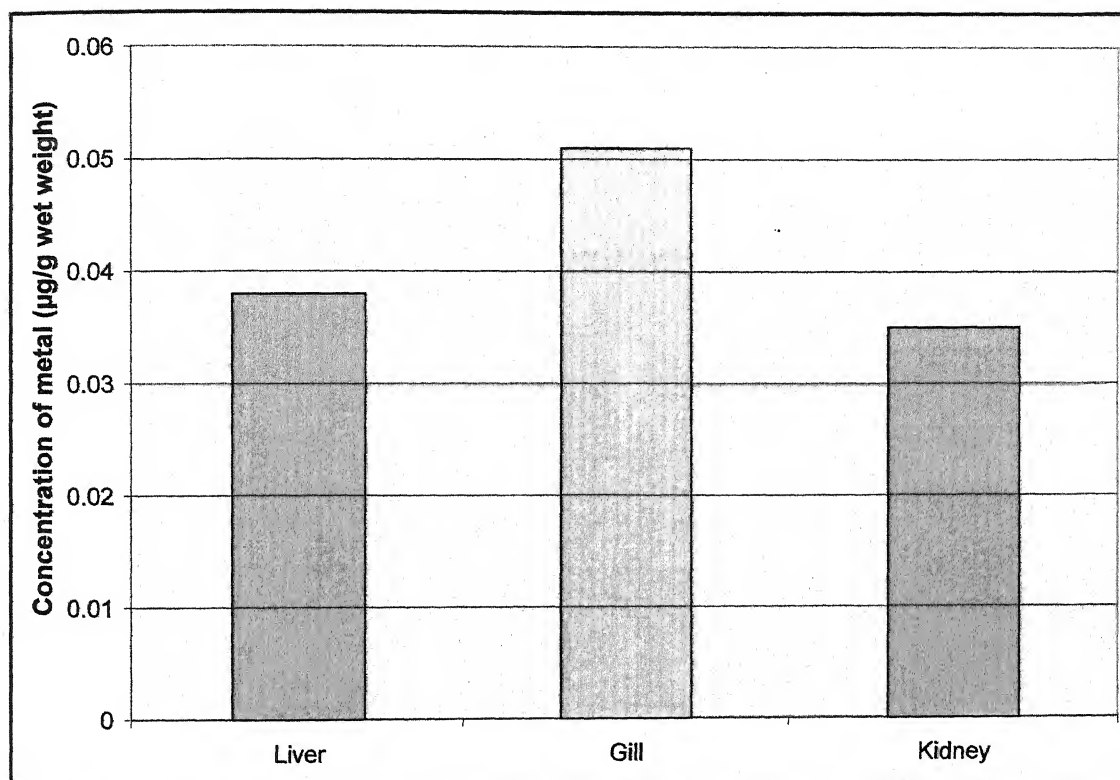
Alteration in growth (weight) of *H. fossilis* (Bloch) in different concentrations of Copper sulphate (mg/L) in 480 h exposure duration.

Fig-51



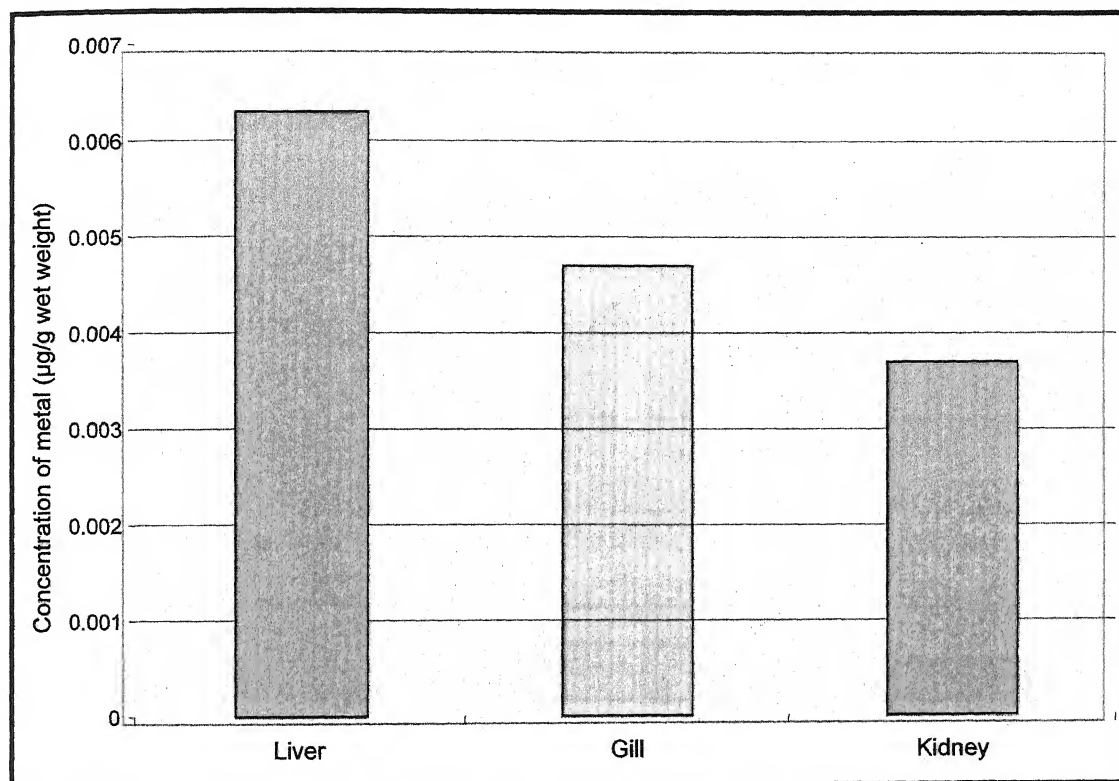
Alteration in growth (weight) of *H. fossilis* (Bloch) in different concentrations of Copper sulphate (mg/L) in 480 h exposure duration.

Fig-51



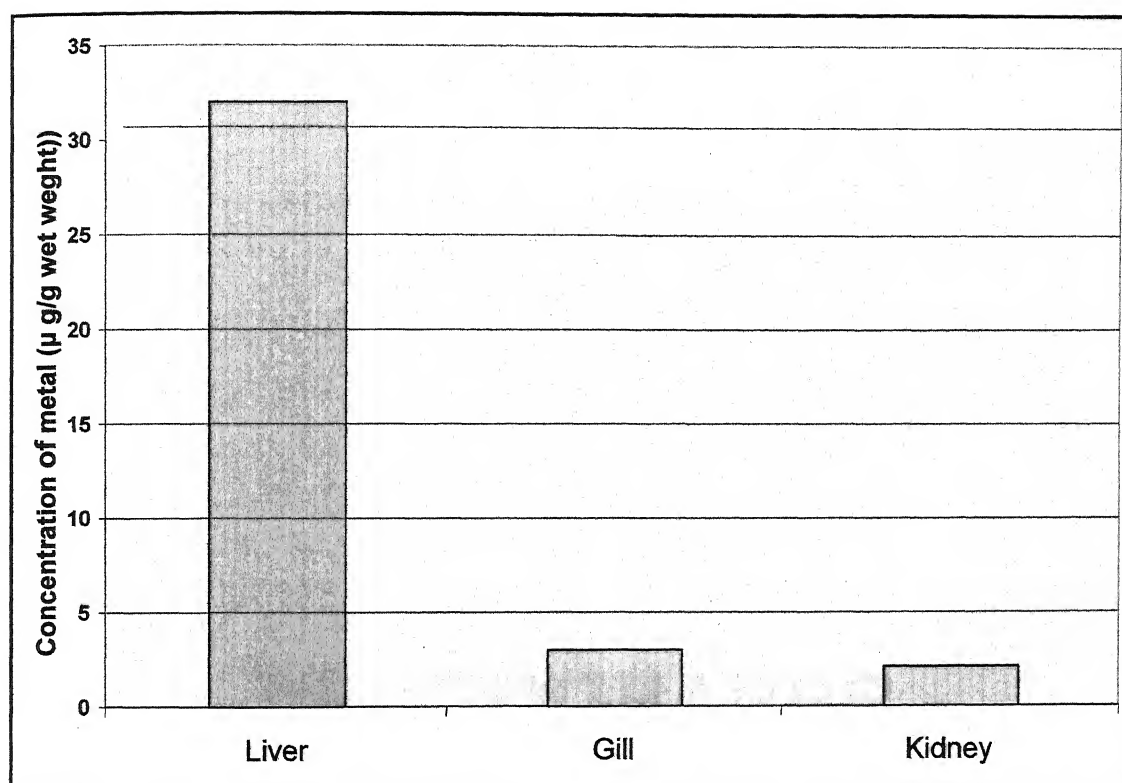
Mean bioaccumulation of cadmium ($\mu\text{g/g}$) in liver, gill and Kidney of *C. carpio* (Linn.) during different exposure period to .32 mg/L Cadmium chloride.

Fig-52



Mean bioaccumulation of Mercury ($\mu\text{g/g}$) in liver, gill and Kidney of *C. carpio* (Linn.) during different exposure period to .038 mg/L Mercuric chloride.

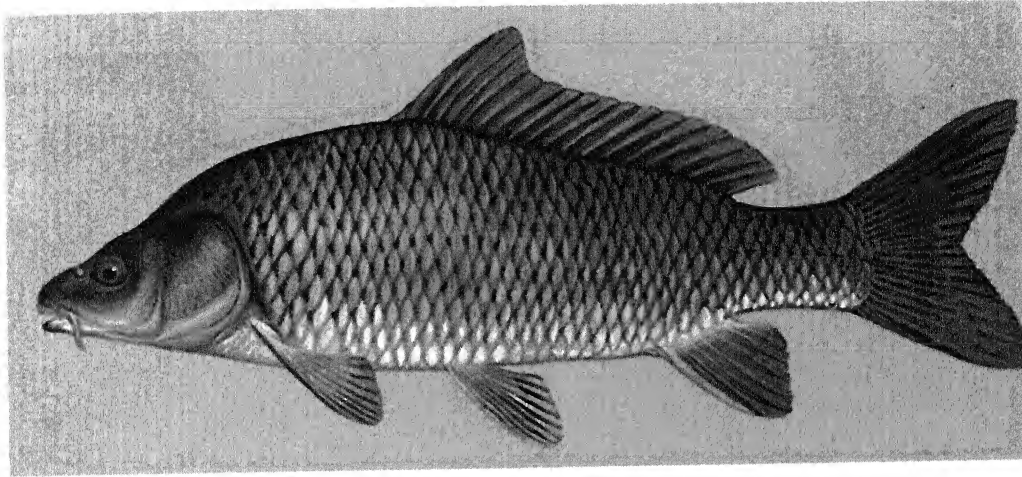
Fig-53



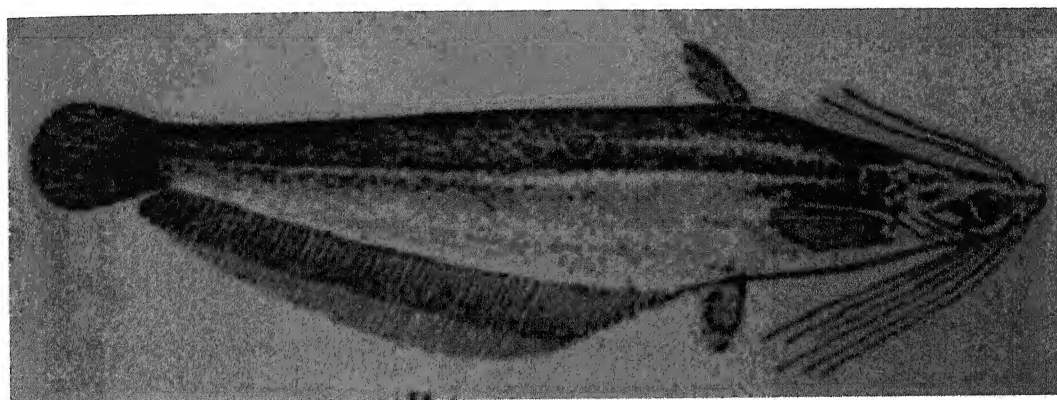
Mean bioaccumulation of Copper ($\mu\text{g/g}$) in liver, gill and Kidney of *H.fossilis* (Bloch) during different exposure period to 2.24 mg/L Copper sulphate.

Fig-54

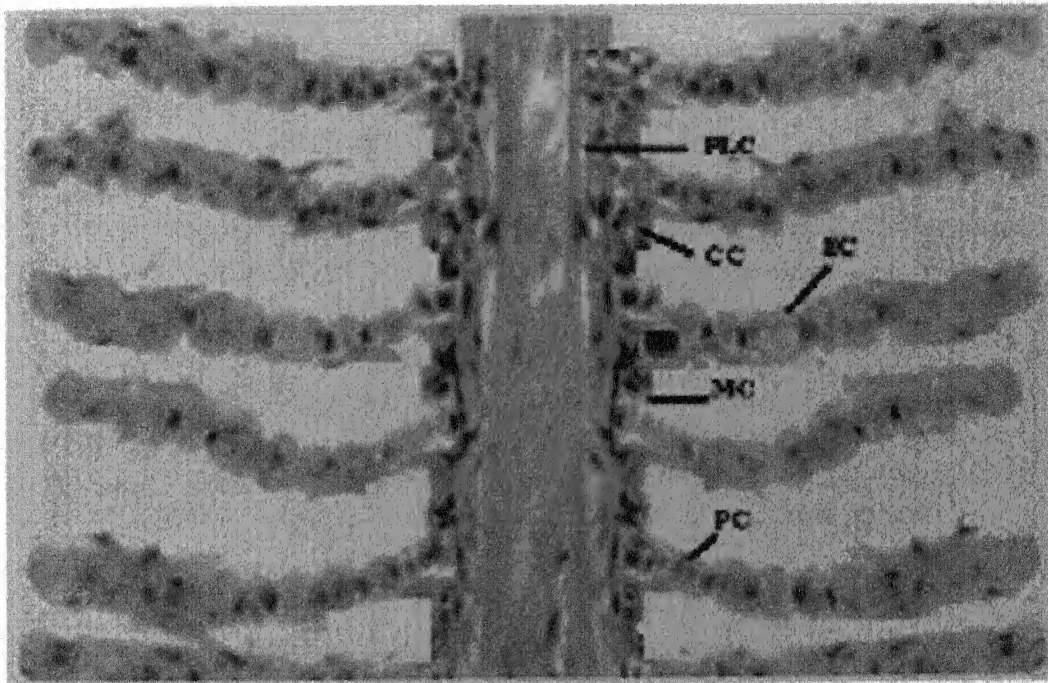
PHOTOGRAPHS



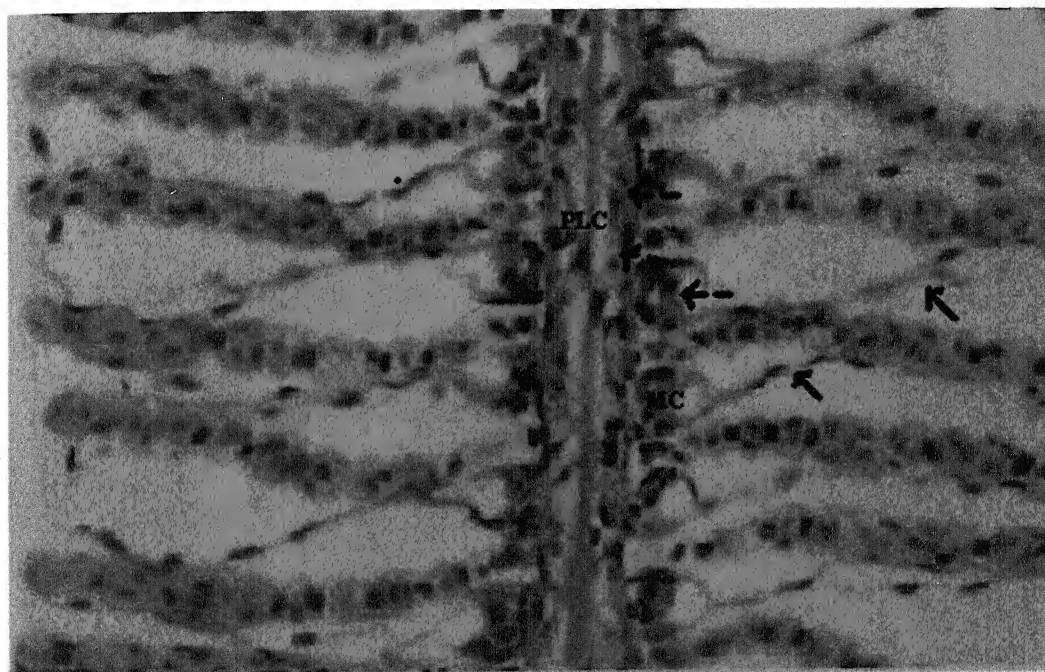
Kingdom	Animalia
Phylum	Chordata
Class	Teleostomi
Subclass	Actinopterygii
Order	Cypriniformes
Family	Cyprinidae
Genus	Cyprinus
Species	<i>carpio</i>



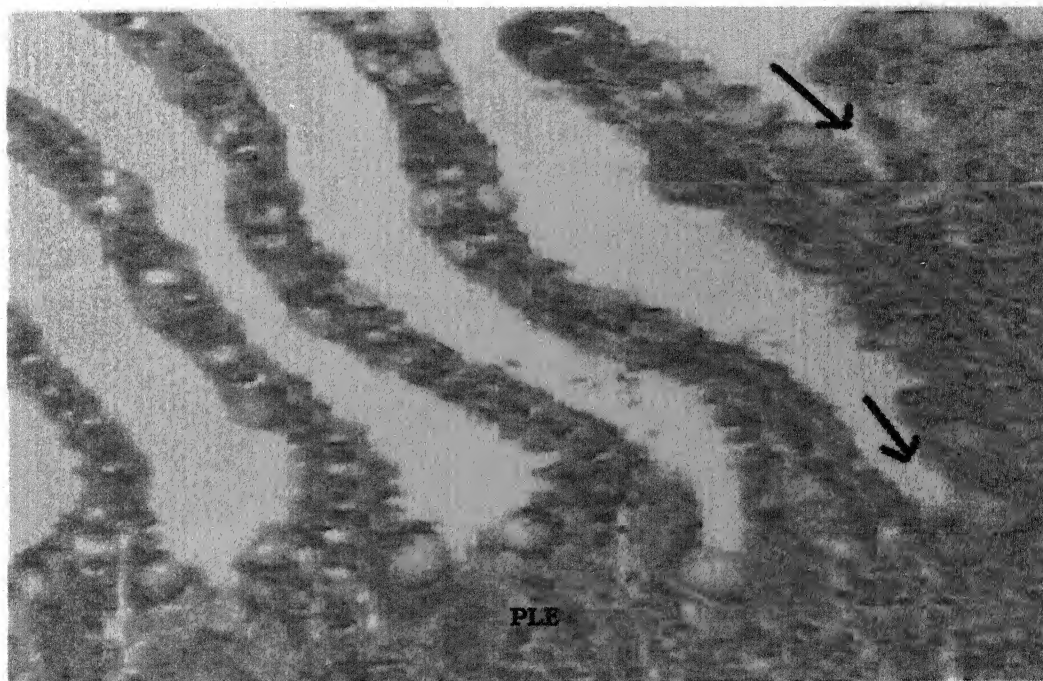
Kingdom	Animalia
Phylum	Chordata
Class	Teleostomi
Subclass	Actinopterygii
Order	Cypriniformes
Family	Siluri
Genus	Heteropneustes
Species	<i>fossilis</i>



Transverse section of gill filament of control fish *C. carpio* showing normal appearance of primary lamellar epithelium (PLE), chloride cells (CC), epithelial cells (EC), mucus cells (MC) & pillar cells (PC).



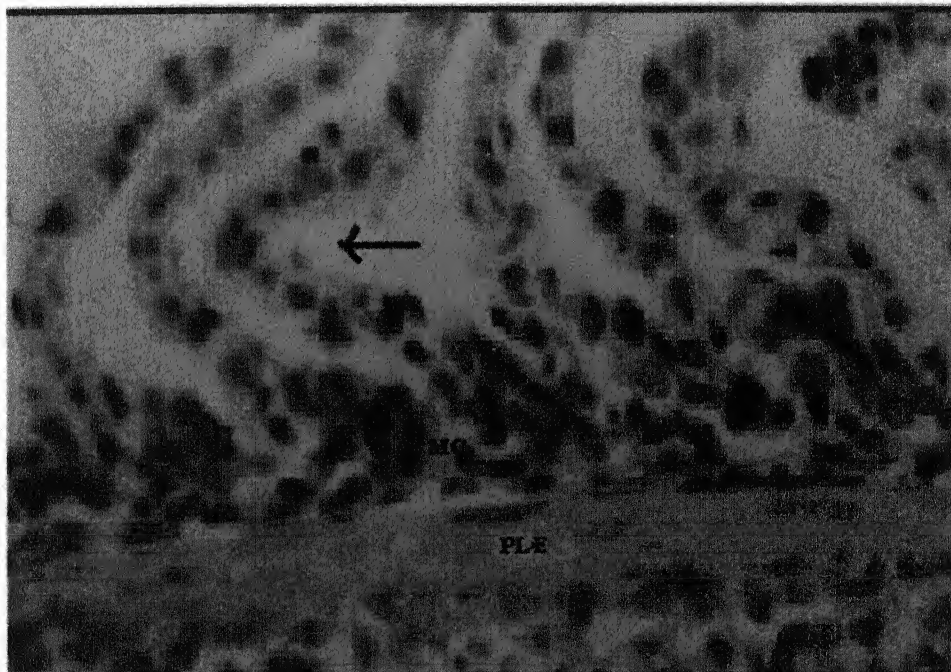
Transverse section of gill filament of 96h treated *C. carpio* to 32 mg/L Cadmium chloride showing primary epithelial lifting (arrows) and thickening of primary lamellar epithelium (PLC) & mucus cell (MC, broken arrow).



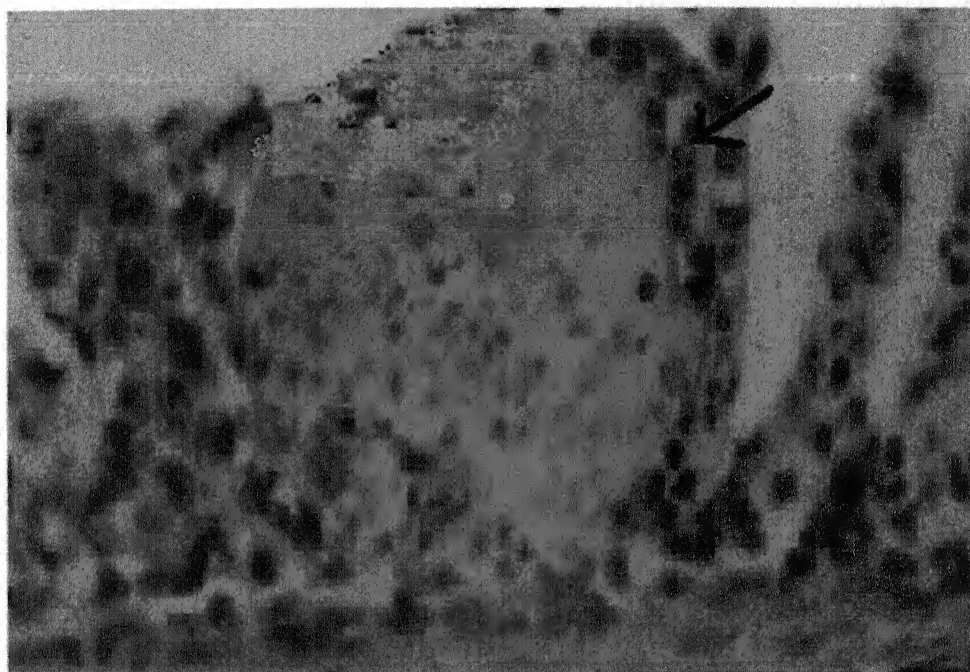
Transverse section of gill filament of 20 days treated *C. carpio* to .32 mg/L Cadmium chloride showing thickening of primary lamellar epithelium (PLE) and fusion of secondary lamellae (arrows).



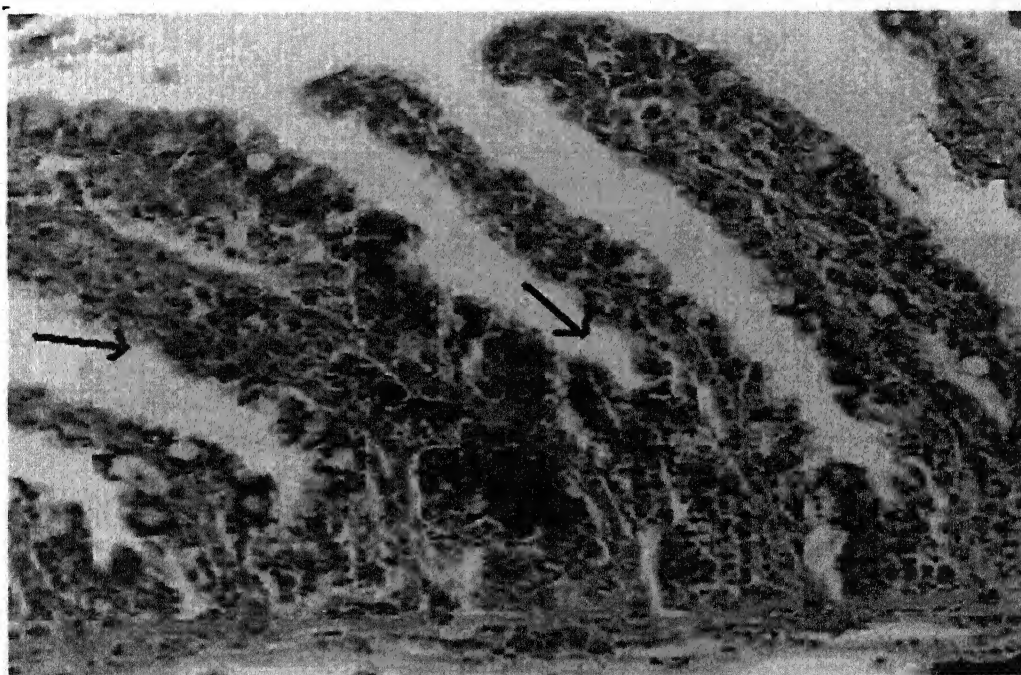
Transverse section of gill filament of 30 days treated *C. carpio* to 1mg/L Cadmium chloride showing secondary lamellar deformity (arrows)



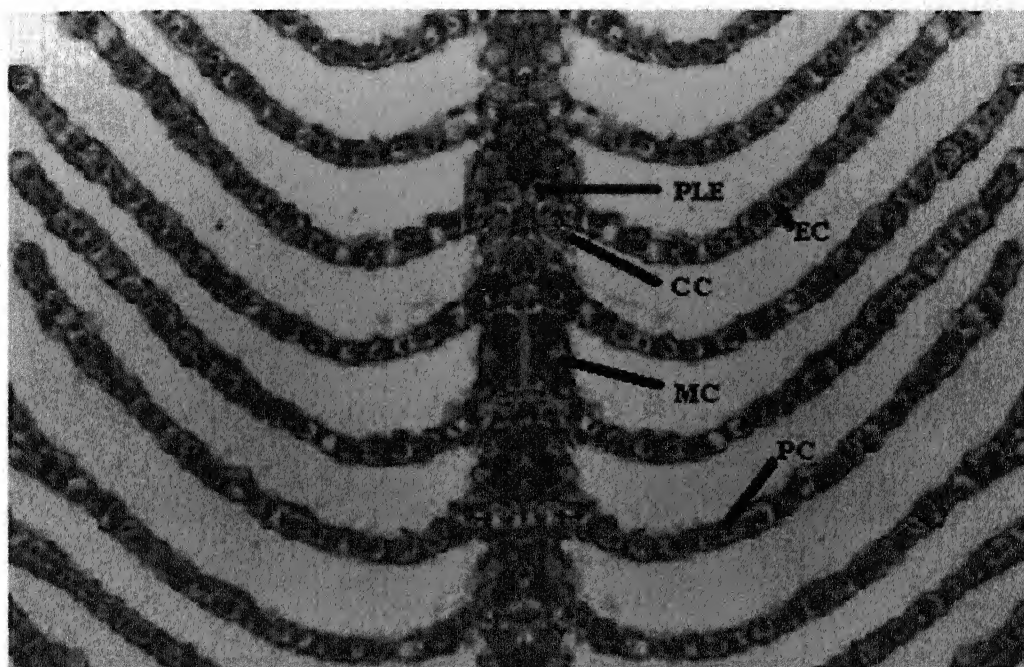
Transverse section of gill filament of 96 hours treated *C. carpio* to .03 mg/L Mercuric chloride showing thickening of primary lamellar epithelium(PLE) , mucus cell (MC) and epithelial lifting (arrow)



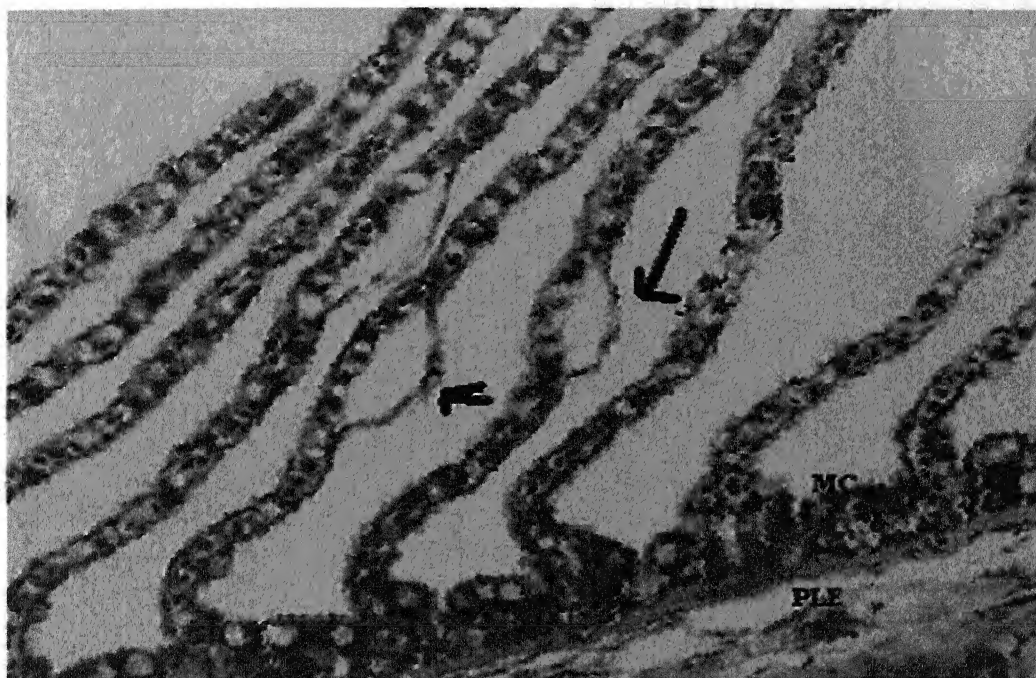
Transverse section of gill filament of 20 days treated *C. carpio* to .03 mg/L Mercuric chloride showing aneurism (arrows) and fusion of lamella.



Transverse section of gill filament of 30 days treated *C. carpio* to .03mg/L Mercuric chloride showing thickening of primary lamella and fusion of mucus cells of secondary lamella (arrow).



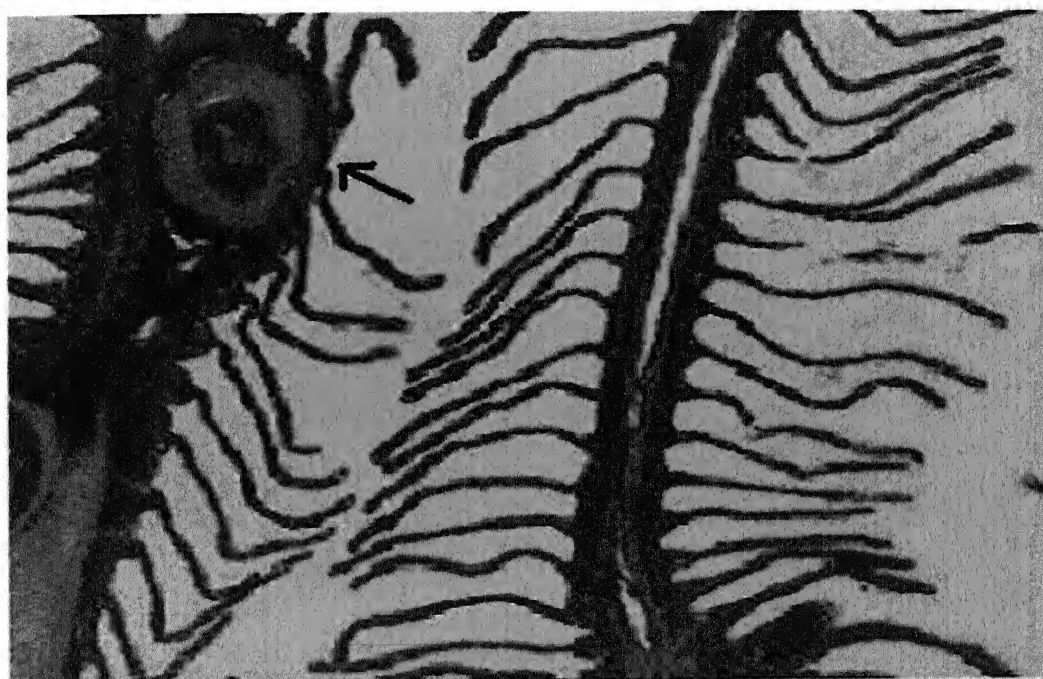
Transverse section of gill filament of control fish *H. fossilis* showing normal appearance of primary lamellar epithelium (PLE), chloride cell(CC) epithelial cells (EC) mucus cells (MC) & pillar cells (PC).



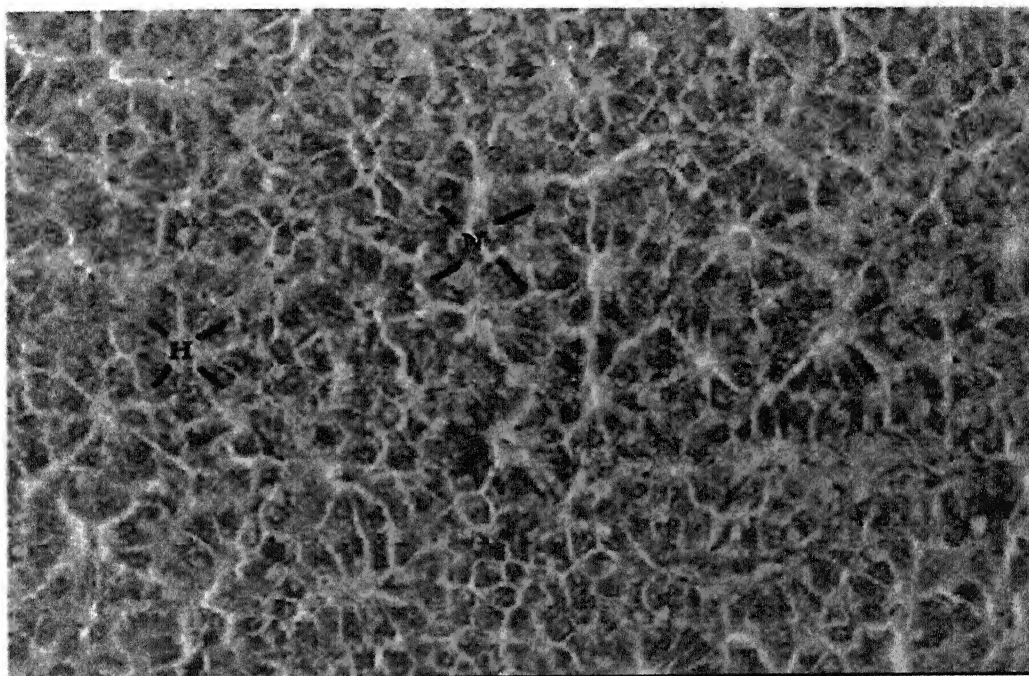
Transverse section of gill filament of 96 h treated *H.fossilis* to 2.24 mg/L Copper sulphate showing thickening of primary lamellar epithelium(PLE), mucus cells (MC) and epithelial lifting (arrow)



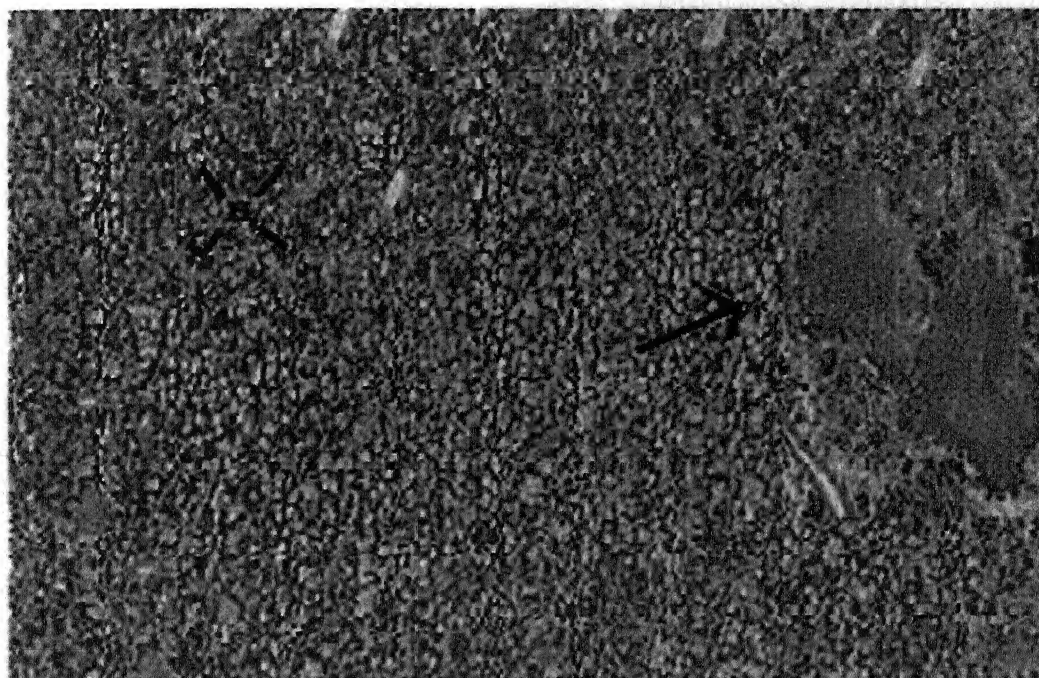
Transverse section of gill filament of 20 days treated *H. fossilis* to 2.24 mg/L Copper sulphate showing thickening of epithelium at the tip of lamellae telangiectasis (arrow) and fusion of secondary lamella (broken arrow).



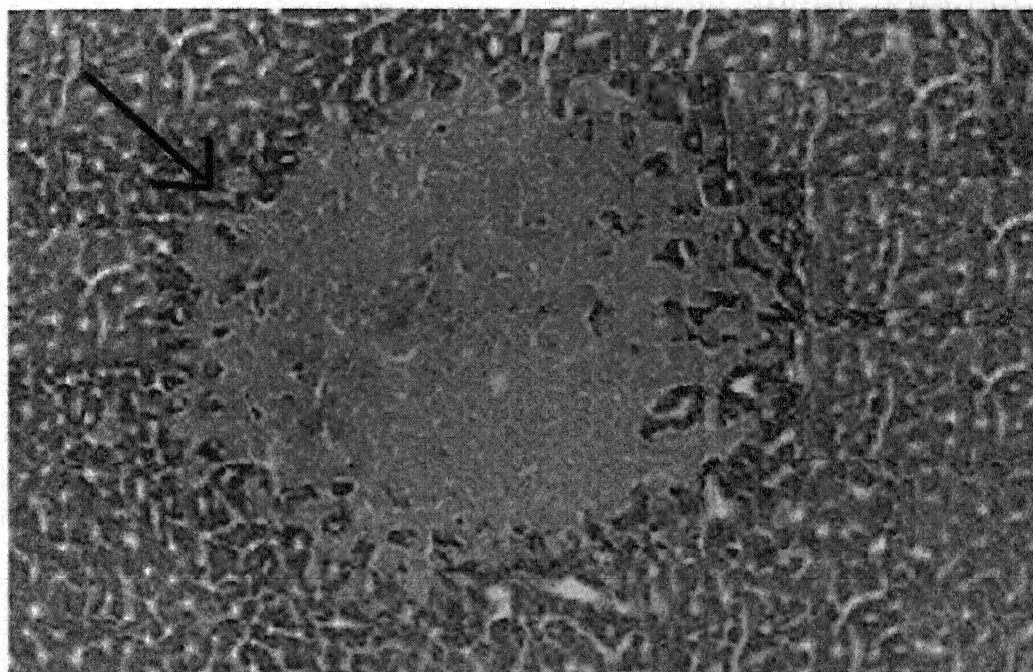
Transverse section of gill filament of 30 days treated *H.fossilis* to 2.24 mg/L Copper sulphate showing tumors (arrow).



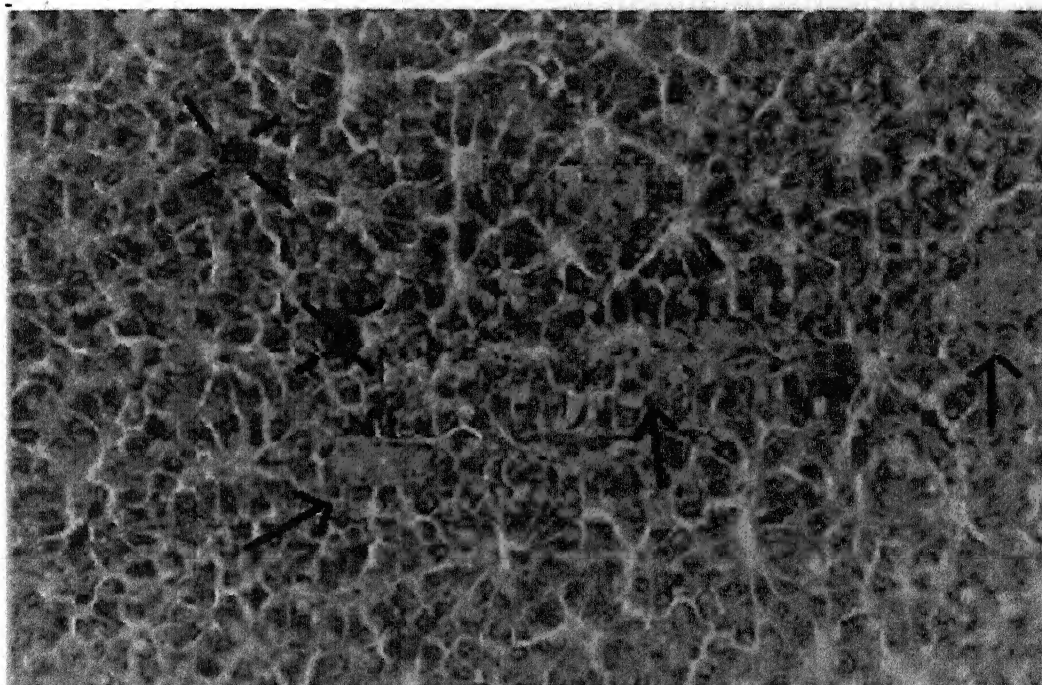
Transverse section of liver tissue of control *C. carpio* showing hepatocytes (H) with central spherical nuclei(N)



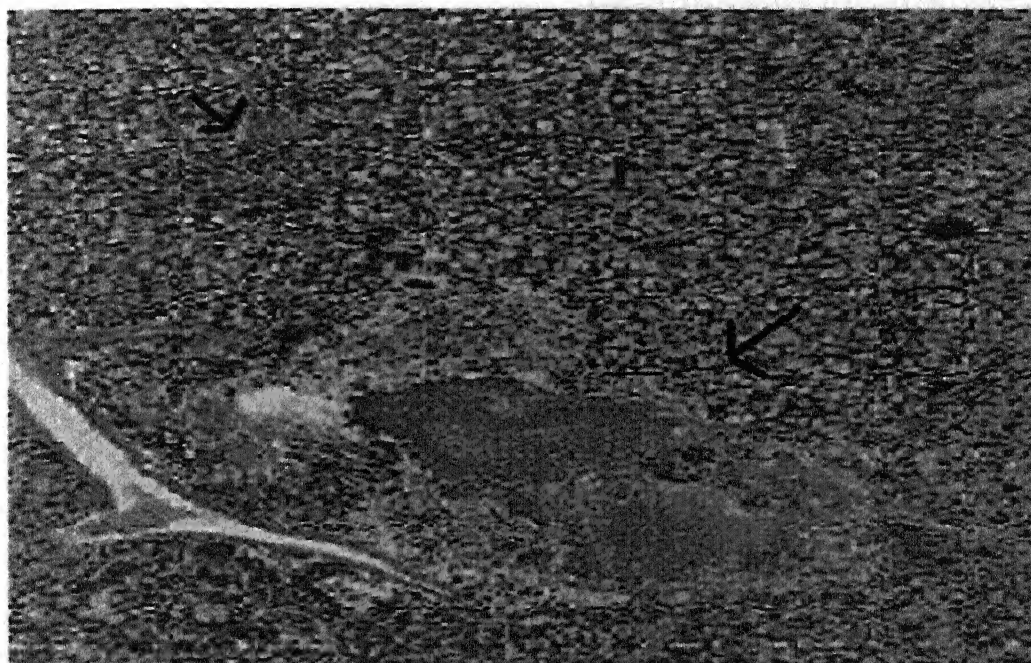
Transverse section of liver tissue of 20 days treated *C. carpio* to .32mg/L Cadmium chloride showing abnormal growth of cells (arrow) & hepatocytes (H).



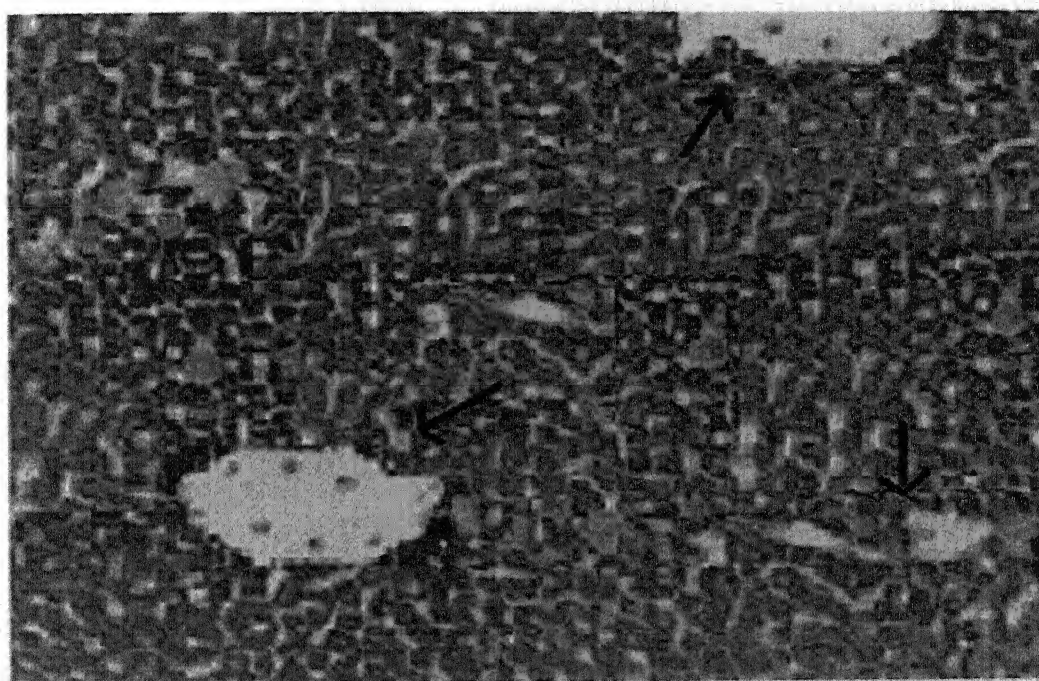
Transverse section of liver tissue of 30 days treated *C.carpio* to 32 mg/L Cadmium chloride showing necrosis (arrow).



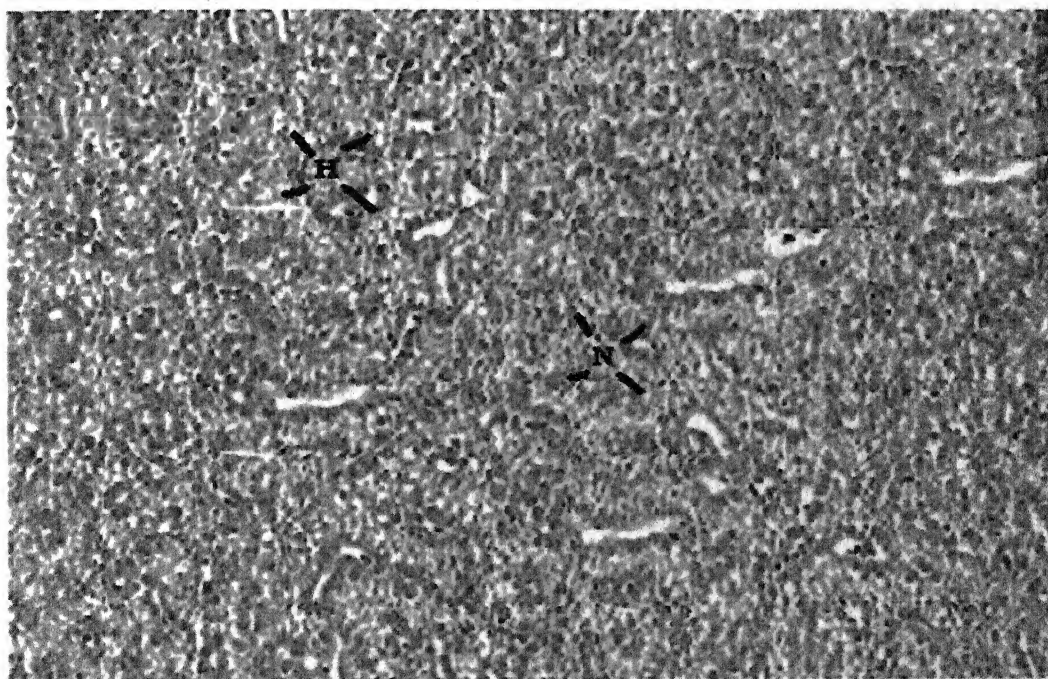
Transverse section of liver tissue of 96h treated *C.carpio* to .03 mg/L Mercuric chloride showing hepatocytes (H) with central spherical nuclei(N), and growing necrosis(arrow)



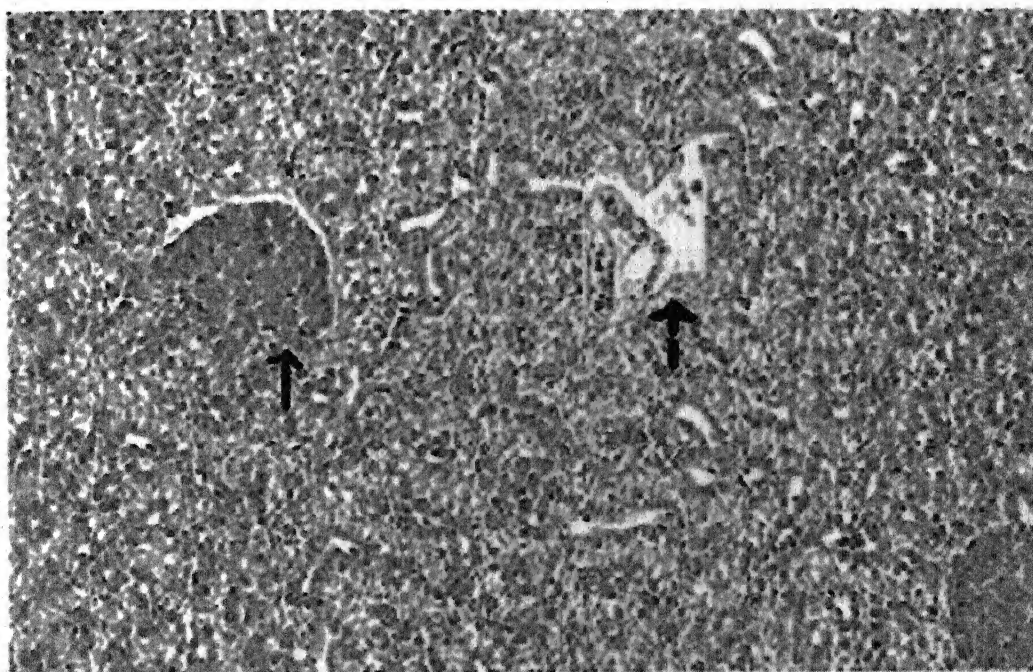
Transverse section of liver tissue of 20 days treated *C. carpio* to .03 mg/L Mercuric chloride showing tumor & irregular clumping (arrow) .



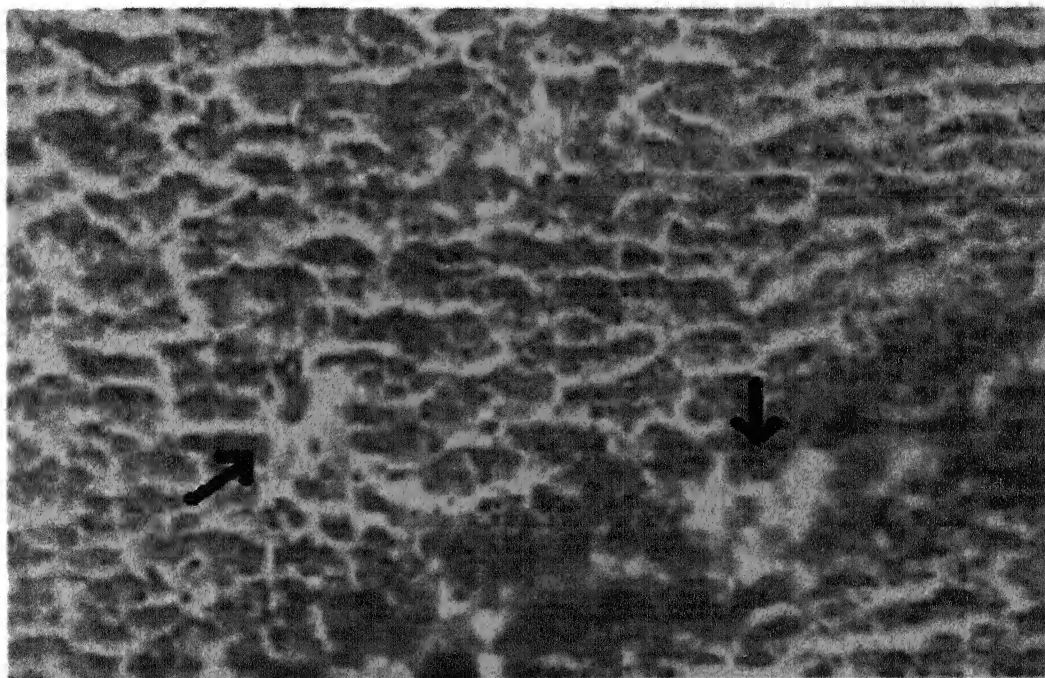
Transverse section of liver tissue of 30 days treated *C.carpio* to .03mg/L Mercuric chloride showing necrosis in hepatocyte (arrow)



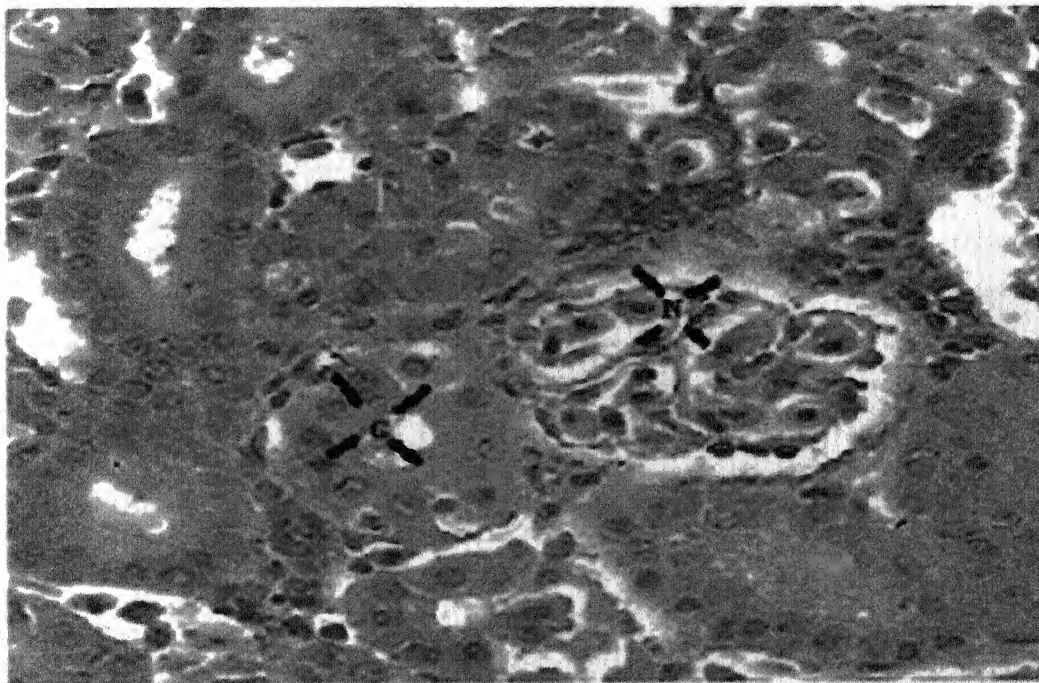
Transverse section of liver tissue of control fish *H. fossilis* showing Hepatocytes (H) with central spherical nuclei (N)



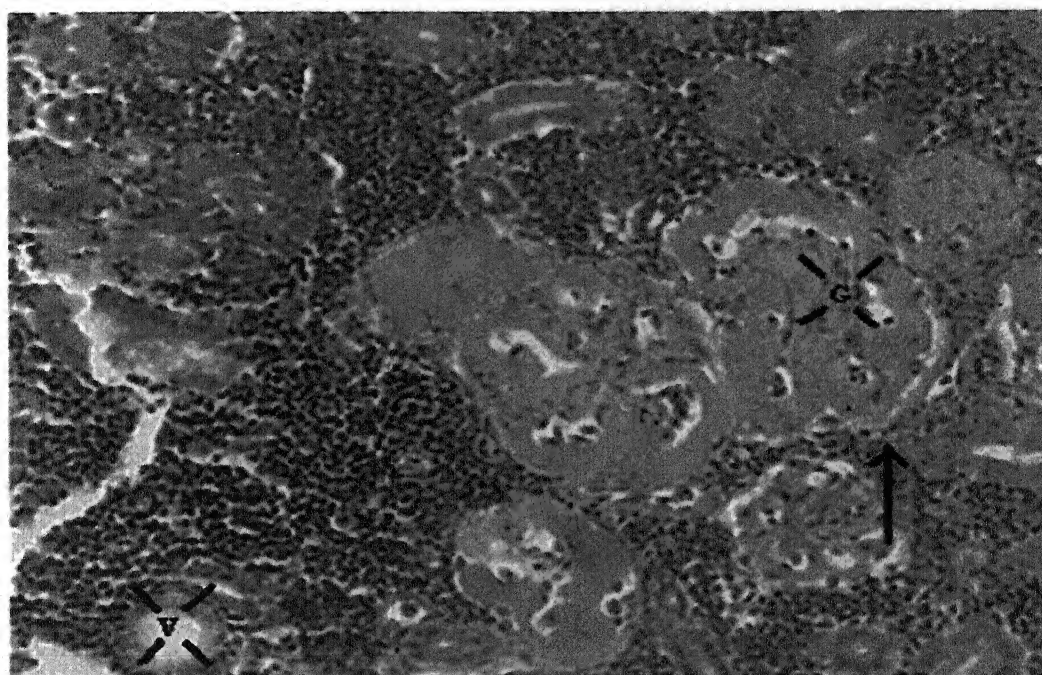
Transverse section of liver tissue of 20 days treated *H. fossilis* to 2.24 mg/L Copper sulphate showing hepatocytes (H) with central spherical nuclei (N) , irregular clumping of cell (arrow) and necrosis (broken arrow)



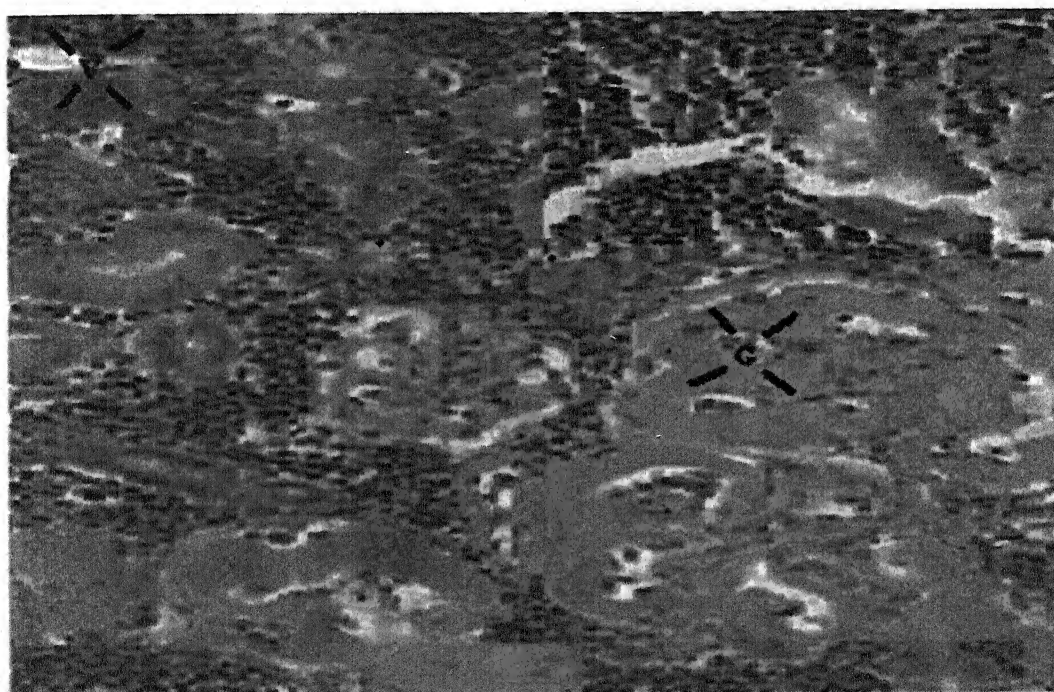
Transverse section of liver tissue of 30 days treated *H. fossilis* to 2.24 mg/L copper sulfate showing abnormal increase in surface area of liver & lysis of cells (arrow).



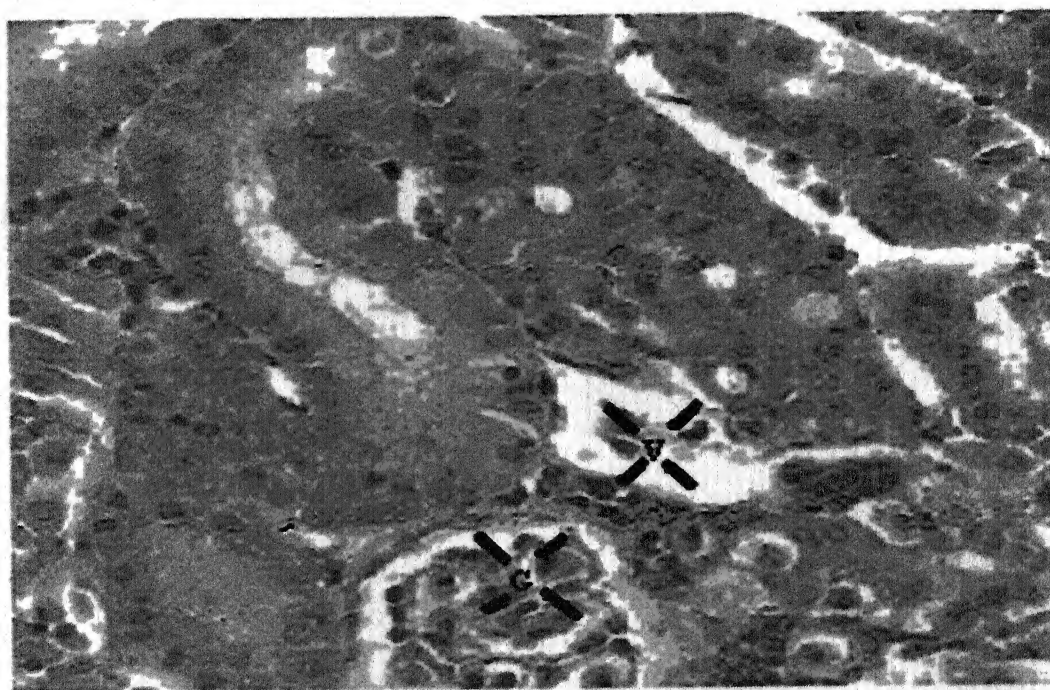
Transverse section of kidney of control *C. carpio* showing normal appearance of glomerular cells with nuclei(N).



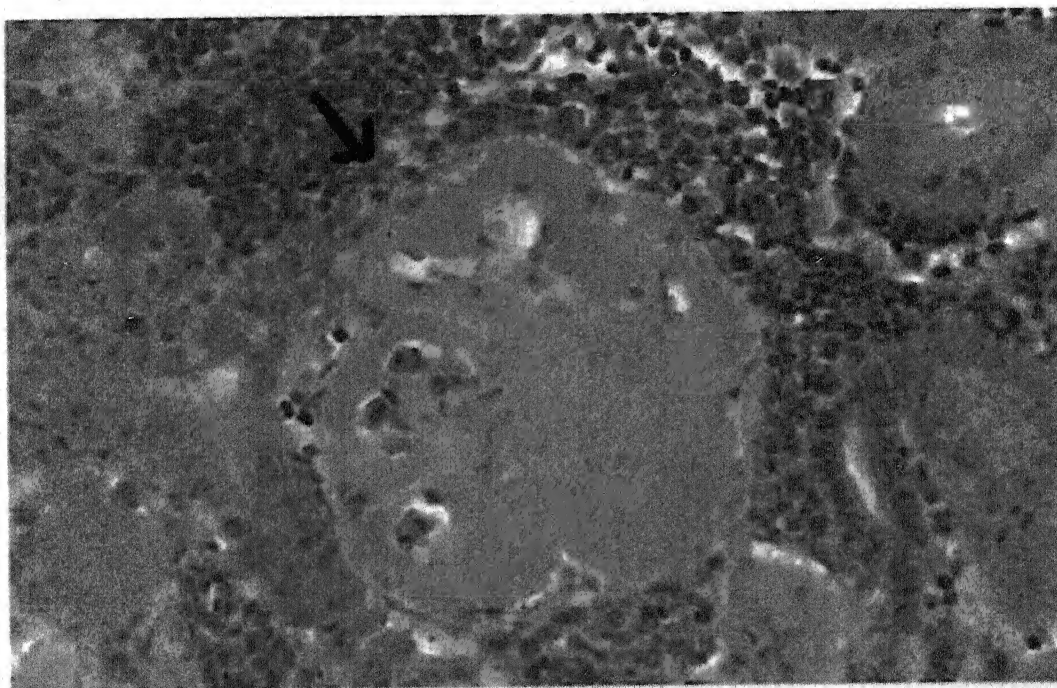
Transverse section of kidney of 20days treated *C.carpio* to .32mg/L Cadmium chloride showing swollen epithelial cells & glomerular(G) distortion & presence of large lipid vacuoles (v)



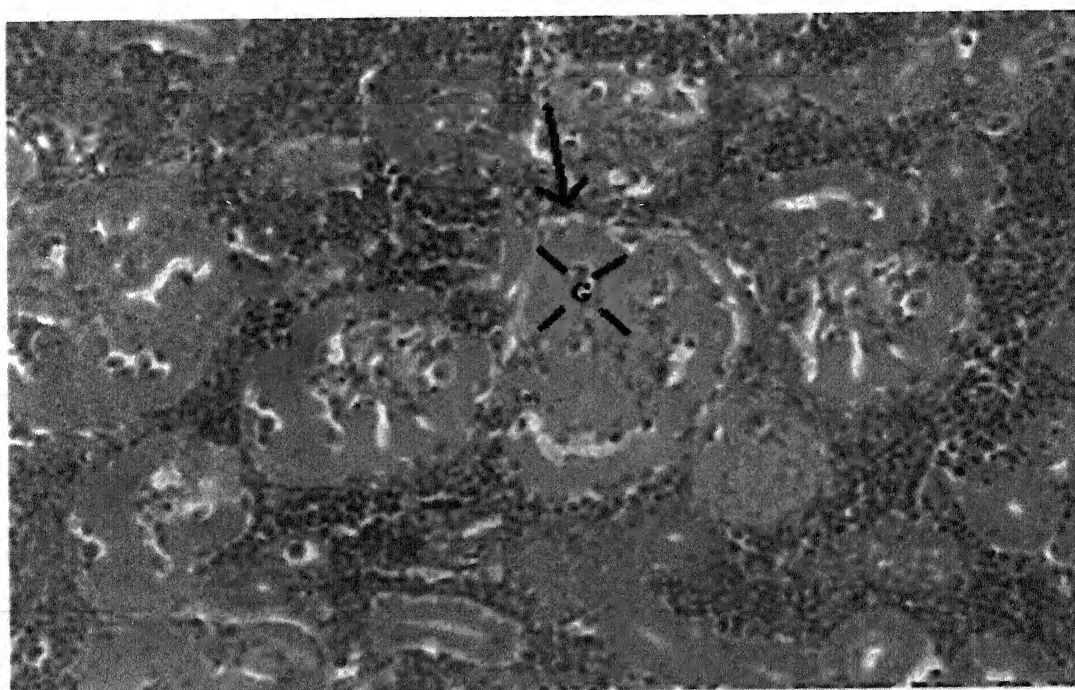
Transverse section of kidney of 30days treated *C.carpio*. to .32 mg/L Cadmium chloride showing swollen epithelial cells & glomerular distortion(G) & presence of large lipid vacuoles (V).



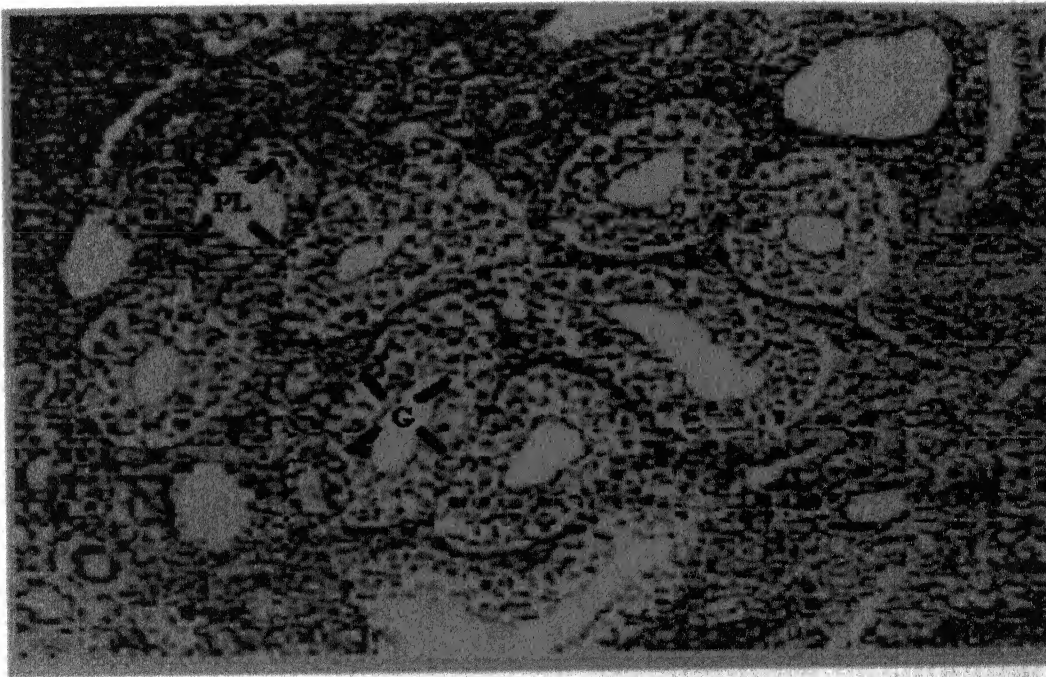
Transverse section of posterior part of kidney of 96h treated *C. carpio* to .03 mg/L Mercuric chloride showing swollen epithelial cells & glomerular distortion(G) & presence of large lipid vacuoles (V).



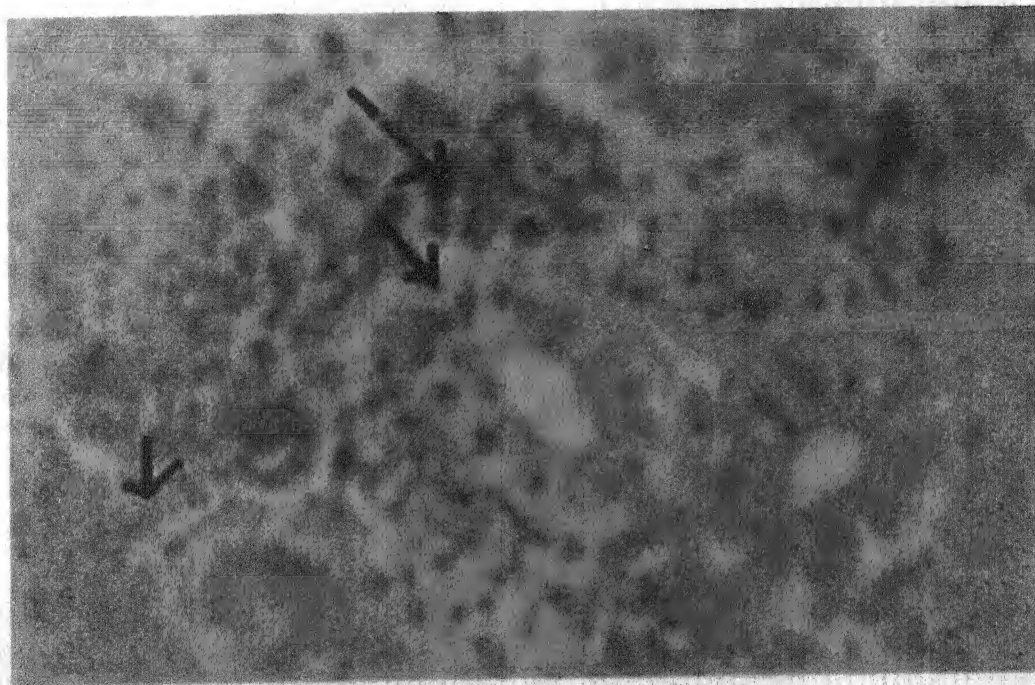
Transverse section of kidney of 20 days treated *C. carpio* to .03 mg/L Mercuric chloride showing glomerular distortion (arrow) & presence of necrosis.



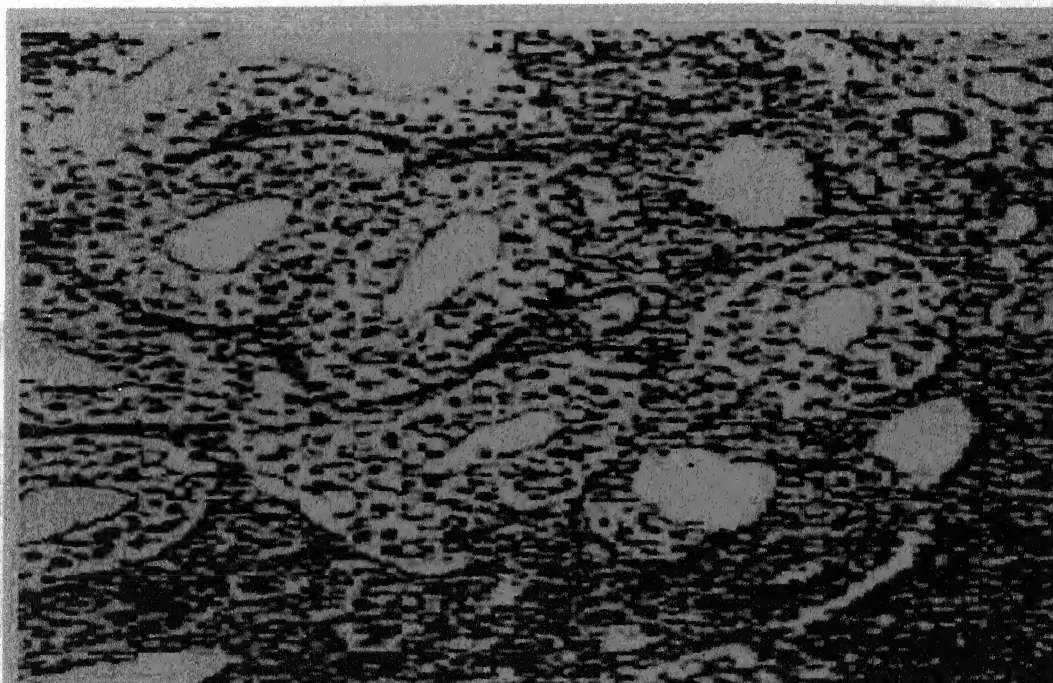
Transverse section of kidney 30days treated *C.carpio* to.03 mg/L
Mercuric chloride showing pycnosis epithelial cells (PL)& glomerular
distortion (arrow).



Transverse section of kidney of control *H.fossilis* showing normal appearance of glomeruli (G) and Proximal tubule (Pl) .



Transverse section of kidney of 20 days treated *H.fossilis* to 2.24mg/L Copper sulphate showing hypertrophy in epithelial cell(broken arrow) and necrosis (arrow)



Transverse section of kidney of 30 days treated *H. fossilis* to 2.24 mg/L Mercuric chloride showing abnormal appearance of glomeruli and proximal tubule.